

ADRENERGIC RECEPTORS ON CEREBRAL CORTICAL NEURONES  
AND THEIR INTERACTION WITH TRICYCLIC  
ANTIDEPRESSANT DRUGS

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### SUMMARY

This thesis describes a series of experiments using the technique of microelectrophoresis. The aim of the experiments was to investigate the pharmacological actions of noradrenaline on single cerebral cortical neurones, and to study the interaction between noradrenaline and tricyclic antidepressant drugs.

1. A series of experiments was conducted on the release of  $^{14}\text{C}$ -labelled noradrenaline from micropipettes in vitro. It was found that (a) in the absence of an electrophoretic current a constant rate of release was established; (b) all retaining currents tested gradually reduced spontaneous release to zero; (c) electrophoretic release was linearly related to the intensity of the ejecting current; (d) retaining currents reduced the amount of noradrenaline released during a subsequent ejection period. These findings are interpreted in terms of a theoretical model of ion movements in micropipettes.

2. The effects of ejecting and retaining currents on the time-course of neuronal responses were investigated. It was found that the kinetics of neuronal responses to microelectrophoretically applied drugs were closely related to the kinetics of drug release.

3. Three kinds of response could be evoked by noradrenaline on cortical neurones: excitatory, depressant and biphasic responses. It was found that the proportions of cells excited and depressed by noradrenaline were related to the baseline firing rates of the neurones studied, a high firing rate increasing the probability of observing depressant responses, and a low firing rate favouring the occurrence of excitatory responses. No correlation could be found



between the direction of the response to noradrenaline and the pH of the noradrenaline solution within the micropipette.

4. The  $\alpha$ -adrenoceptor stimulating agents phenylephrine and methoxamine evoked only excitatory responses, whereas the  $\beta$ -adrenoceptor agonist salbutamol had only depressant effects. Isoprenaline, similarly to noradrenaline, could evoke both depressant and excitatory responses: while lower doses evoked depressant responses, higher doses were usually excitatory on the same cells. Excitatory responses could be selectively antagonised by the  $\alpha$ -adrenoceptor blocking agents phentolamine and phenoxybenzamine, whereas the depressant responses could be selectively antagonised by the  $\beta$ -adrenoceptor blocking agent sotalol. These findings suggest that both  $\alpha$ - and  $\beta$ -adrenoceptors occur on cortical neurones, and that these receptors mediate opposite effects, the  $\alpha$ -receptors being excitatory, and the  $\beta$ -receptors being inhibitory.

5. Dopamine, similarly to noradrenaline, could evoke both excitatory and depressant responses on cortical neurones; there was a high correlation between the direction of responses to dopamine and noradrenaline. Dopamine tended to evoke smaller responses than noradrenaline, although the transport number of dopamine was somewhat higher than that of noradrenaline. Both the  $\alpha$ -adrenoceptor blocking agent phenoxybenzamine, and the neuroleptics haloperidol and  $\alpha$ -flupenthixol could discriminate between excitatory responses to dopamine and noradrenaline: while responses to dopamine were more sensitive to the antagonistic effects of neuroleptics, responses to noradrenaline were more susceptible to the blocking effect of phenoxybenzamine. These observations suggest the existence of two separate excitatory catecholamine receptors: an  $\alpha$ -adrenoceptor and a dopamine receptor.

6. The noradrenaline precursor L-dihydroxyphenylalanine (DOPA) mimicked the effects of noradrenaline: each cell excited by DOPA, was also excited by noradrenaline, and each cell depressed by DOPA was also depressed by noradrenaline. DOPA appeared to be less potent than noradrenaline, and evoked responses of a slower time-course than noradrenaline. Responses to DOPA could be antagonised by the adrenoceptor blocking agents phentolamine and propranolol. These results are consistent with the hypothesis that the responses to DOPA are mediated via adrenoceptors.

7. The tricyclic antidepressant drugs imipramine and desipramine had a dual effect on both excitatory and depressant responses to noradrenaline: while smaller doses potentiated, bigger doses antagonised the responses. It is suggested that potentiation may reflect the blockade of uptake of noradrenaline into presynaptic noradrenergic terminals, whereas antagonism may reflect the direct post-synaptic adrenoceptor blocking properties of tricyclic antidepressants.

8. Responses to noradrenaline could also be potentiated by iprindole, a tricyclic antidepressant with no uptake blocking activity. This observation would suggest the existence of a postsynaptic mechanism for potentiation.

9. Responses to mescaline could also be potentiated by desipramine, although this antidepressant does not block the uptake of mescaline into brain tissue. This finding would suggest the existence of a postsynaptic mechanism for potentiation.

10. The indirectly acting sympathomimetic amine tyramine could evoke both excitatory and depressant responses on cortical neurones. There was a high correlation between the direction of responses to tyramine and noradrenaline. Tyramine appeared to be less potent than noradrenaline, although there was no significant difference between the transport numbers of the two amines. Tyramine evoked 'slower' responses than noradrenaline. Desipramine could discriminate between responses to tyramine and noradrenaline: while responses to tyramine were antagonised, responses to noradrenaline were either potentiated or unaffected. These results are consistent with the hypothesis that tyramine acts by releasing noradrenaline from presynaptic stores, and that desipramine blocks the uptake of both tyramine and noradrenaline.

11. A theoretical model of 'antagonistic agonism' is developed to describe the action of an agonist at two functionally antagonistic receptor populations within the same pharmacological test system. The model is applied to the case of functionally antagonistic  $\alpha$ - and  $\beta$ -adrenoceptors on cortical neurones. The possible functional significance of 'opposite receptors' is discussed.

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CHAPTER I

GENERAL INTRODUCTION

This thesis examines the pharmacology of neuronal adrenoceptors in the cerebral cortex, and also investigates how tricyclic antidepressant drugs interact with these receptors. The thesis consists of eleven chapters which can be divided into three groups: Chapters II and III deal with some methodological aspects of the microelectrophoretic technique; Chapters IV - VII examine the pharmacology of neuronal adrenoceptors; Chapters VIII - XI are devoted to the neuronal pharmacology of tricyclic antidepressant drugs. Since the experimental evidence presented in Chapters IV - VII suggests that noradrenaline may activate functionally antagonistic receptors on the same neurone, an Appendix is included which examines the quantitative pharmacology of such a test system.

The aim of this General Introduction is to briefly summarize our state of knowledge in the fields most relevant to this thesis. Since each Chapter has its own introduction, no attempt is made here to give a comprehensive review of the literature.

### I.1. ADRENOCEPTORS

Noradrenaline (NA) is the natural transmitter substance of peripheral sympathetic neurones, and adrenaline is the hormone released by gland cells in the adrenal medulla. Both noradrenaline and adrenaline evoke distinct pharmacological changes in peripheral tissues (smooth muscle cells, gland cells). The pharmacological receptors mediating these responses are referred to as adrenoceptors (or adrenergic receptors) (see Furchgott, 1972; Burnstock & Costa, 1975; Triggle & Triggle, 1976).

It is usual to distinguish between two major classes of adrenoceptors:  $\alpha$ -receptors and  $\beta$ -receptors (Ahlquist, 1948). The distinction between these two classes of adrenoceptor is based on the relative potencies of various adrenergic agonists and on the susceptibility to blockade by specific adrenergic antagonists.

$\alpha$ -adrenoceptors are characterized by the following relative molar potency series of agonists: adrenaline  $\geq$  noradrenaline  $>$  phenylephrine  $>$  isoprenaline; these receptors can be specifically blocked by phentolamine, dibenamine, or phenoxybenzamine.  $\beta$ -adrenoceptors are characterized by the following relative potency series of agonists: isoprenaline  $>$  adrenaline  $>$  noradrenaline  $>$  phenylephrine, or: isoprenaline  $>$  noradrenaline  $>$  adrenaline  $>$  phenylephrine; these receptors can be blocked by relatively low concentrations of propranolol or pronethalol. Recently subtypes of  $\beta$ -receptors ( $\beta_1$  and  $\beta_2$ ) have been differentiated (see Furchgott, 1972).

## I.2. THE NORADRENERGIC SYSTEM OF THE BRAIN

With the use of the Falck-Hillarp fluorescent histochemical technique, Dahlström & Fuxe (1965) and Fuxe (1965) demonstrated a noradrenergic neuronal system in the brain. The NA-containing cell bodies are localized in distinct nuclei of the lower brain stem, and project diffusely to most parts of the neuraxis. The terminals of the NA-containing neurones arborize similarly to peripheral vegetative neurones creating characteristic 'varicosities' (cf. the sympathetic ground plexus or terminal network; see Burnstock & Costa, 1975).

There are three groups of NA-containing cell bodies in the brain stem (Fig. 1/I): 1. caudal group (in the lateral reticular formation of the medulla oblongata); 2. oral group (in the reticular formation of the medulla and the pons); 3. locus coeruleus (Dahlström & Fuxe, 1965; Ungerstedt, 1971). The caudal group gives rise to a descending system of fibres which innervate neurones in the ventral and lateral horns of the spinal cord. The oral cell group gives rise to two fibre tracts: 1. short axons to cells in the mid-brain reticular formation; 2. the ventral ascending noradrenaline-bundle to the hypothalamus and the limbic system. The locus coeruleus (a small cell group on the floor of the fourth ventricle) is the most important of the NA-containing cell groups. Three important tracts originate from this nucleus: 1. descending pathway to nuclei in the lower brain stem; 2. lateral pathway to the cerebellum; 3. ascending pathway ('dorsal ascending noradrenaline bundle') to the hypothalamus, limbic system (especially hippocampus), and neocortex. There is evidence suggesting that one single NA-containing neurone in the locus coeruleus can send axon collaterals to three different structures (to the neocortex, hippocampus, and cerebellum) (Ungerstedt, 1971).

Recently it has been demonstrated that the majority of the noradrenergic varicosities do not form synaptic contacts: they are located in gaps between glia cells and nerve cells, and the released noradrenaline probably affects a larger number of neurones situated in the vicinity of the site of release (Descarries et al., 1977). This would suggest a localized hormone-like action for NA in the brain (Dismukes, 1977).

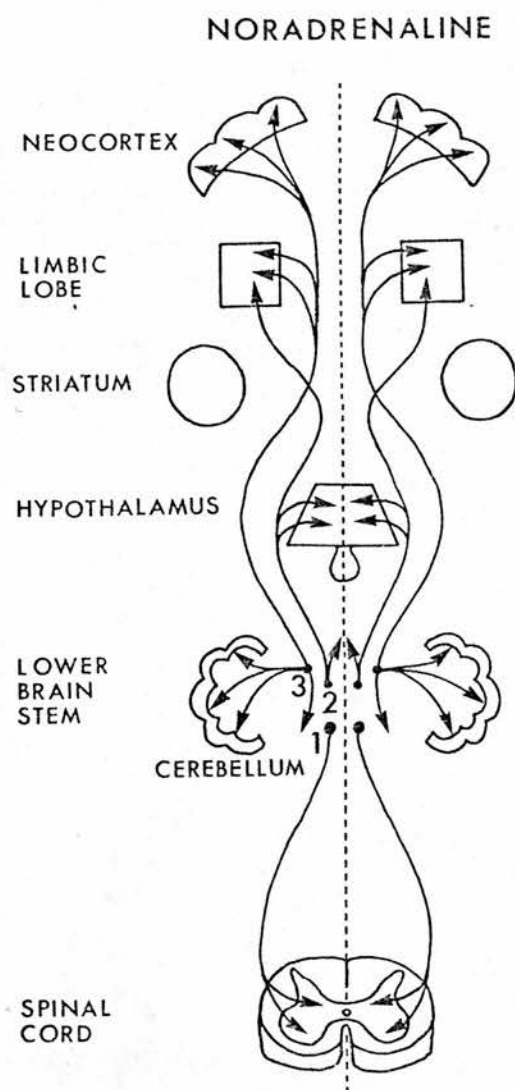


FIGURE 1/I. Schematic diagram of the noradrenaline-containing neuronal system.

1: caudal cell group and the descending noradrenaline bundle;  
 2: oral cell group and the ventral ascending noradrenaline bundle;  
 3: locus coeruleus and its projections to the brain stem, to the cerebellum, and to the limbic lobe and neocortex (dorsal ascending noradrenaline bundle).

### 1.3. THE TECHNIQUE OF MICROELECTROPHORESIS

The technique of microelectrophoresis enables the investigator to apply drugs into the immediate vicinity of central neurones, whilst recording the electrical activity of the neurone under study. This technique was first developed by Nastuk (1953) and Castillo & Katz (1955; 1957) for the study of muscle end-plate pharmacology; later the technique was adopted by Curtis and his colleagues (see Curtis, 1964) for the study of the pharmacology of single central neurones.

For the study of central neurones, multibarrelled micropipettes are usually used: one barrel is filled with a concentrated sodium chloride solution for the recording of action potentials, whereas the remaining barrels contain ionized drug solutions. A schematic diagram of the experimental 'set-up' is shown in Fig. 2/I; for details see II.2. and III.2. Drug molecules are ejected from the micropipette, or retained within the pipette by the passage of currents of appropriate polarity. Action potentials can be displayed on the screen of an oscilloscope (which can be filmed for permanent records), can be stored on tape, or can be converted into a ratemeter write-out (see Fig. 3/I; for details see III.2.).

There are detailed reviews describing several aspects of the microelectrophoretic technique (Curtis, 1964; Kelly, Simmonds & Straughan, 1975; Kelly, 1975). Chapters II and III of this thesis deal with some quantitative aspects of the technique: Chapter II examines the physical processes underlying the release of drugs

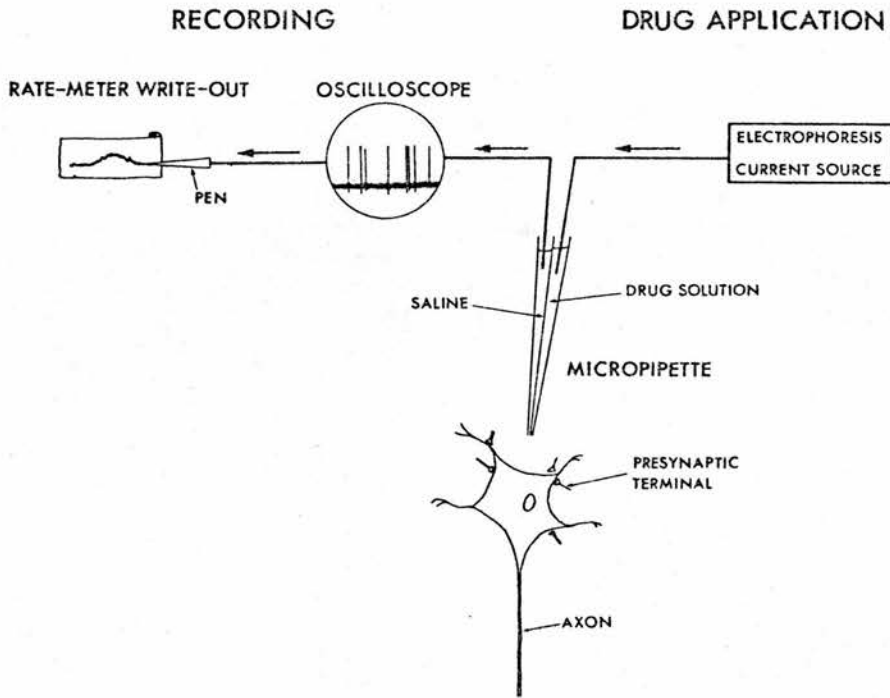


FIGURE 2/I. Schematic diagram showing the experimental 'set-up' used in microelectrophoresis experiments.

Only two barrels of a multi-barrelled micropipette are shown. The tip of the micropipette is in the proximity of a central neurone. On the left, the recording barrel, which is filled with a saline solution, is connected to an oscilloscope and to a ratemeter. On the right, the 'drug barrel' is connected to an electrophoretic current source.

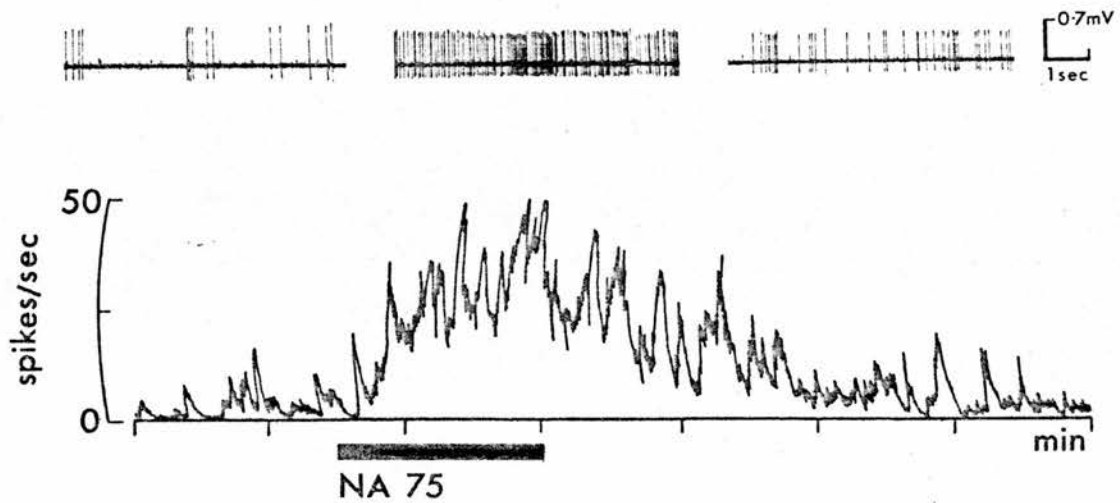


FIGURE 3/I. The excitatory response of a single neurone in the cerebral cortex of the cat to noradrenaline applied by microelectrophoresis.

The top of the figure shows excerpts from the oscilloscope photographs of the firing of the neurone. Vertical deflections correspond to individual action potentials. On the left: before noradrenaline was applied; in the middle: during the application of noradrenaline; on the right: after the application of noradrenaline.

The bottom of the figure shows the whole excitatory response in the form of a ratemeter write-out. Ordinate: firing rate (spikes/sec); abscissa: running time (min). The horizontal bar indicates the microelectrophoretic application of noradrenaline; the number refers to the intensity of the ejecting current in nanoamperes (nA).



(e.g. noradrenaline) from micropipettes; Chapter III examines the relationship between the kinetics of the release of drugs from micropipettes and the time-course of neuronal responses.

#### I.4. NEURONAL RESPONSES TO NORADRENALINE

The use of the microelectrophoretic technique has revealed that most parts of the mammalian central nervous system contain neurones which are sensitive to NA (for reviews see Curtis & Crawford, 1969; Krnjevic; 1974; Tebėcis, 1974).

##### I.4.1. SPINAL CORD

###### a. Interneurones and Renshaw cells

Curtis et al. (1961) found that NA was without an effect on neurones in the spinal cords of cats anaesthetised with barbiturates. Later, however, both depressant and excitatory actions of NA were described on spinal interneurones.

Engberg & Ryall (1966) and Curtis et al. (1971) described a depressant action of NA on spinal interneurones and Renshaw cells; these responses could be blocked by strychnine while responses to  $\gamma$ -aminobutyric acid were not affected (Curtis et al., 1971). Weight & Salmoiraghi (1966) observed both excitatory and depressant responses of spinal interneurones to NA applied by microelectrophoresis. Biphasic responses (consisting of an initial depressant phase followed

by an excitation) also occurred in their material. Similarly, in a recent paper, Headley & Lodge (1976) described two kinds of responses to NA on spinal interneurons and Renshaw cells: monophasic depressant responses and biphasic responses consisting of a depressant phase followed by an excitatory phase. These authors suggested that NA might activate both inhibitory and excitatory receptors on spinal neurones.

#### b. Motoneurons

Engberg & Marshall (1971) examined the effects of micro-electrophoretically applied NA on the membrane potential of motoneurons, using intracellular recording. These authors found that NA hyperpolarized the motoneurone membrane; this hyperpolarization was accompanied by a decrease in membrane conductance. More recently, Engberg et al. (1976) concluded that the membrane hyperpolarization of motoneurons evoked by NA probably was not mediated via specific adrenoceptors, since a wide range of different agents (adrenergic agonists, antagonists, and neuroleptics) evoked very similar effects. In contrast to the reports of Engberg and his colleagues, Barasi & Roberts (1977) found an excitatory action of NA on motoneurons: NA increased the amplitude of field potentials originating from antidromically activated motoneurons. These authors concluded that NA, similarly to 5-hydroxytryptamine and dopamine, increased the excitability of motoneurons.

#### I.4.2. BRAIN STEM

##### a. Reticular formation

Straschill & Perwein (1971) reported a depressant action of NA on neurones in the mesencephalic reticular formation. However, most investigators described both excitatory and depressant responses of neurones to NA in the brain stem reticular formation (Boakes et al., 1970; Boakes et al., 1971; Hösli et al., 1971; Anderson et al., 1973). Biphasic responses were also observed.

The excitatory and depressant responses of brain stem neurones were not affected by conventional  $\alpha$ - and  $\beta$ -adrenoceptor blocking agents (Boakes et al., 1971). On the other hand, the excitatory responses could be blocked by chlorpromazine (Bradley et al., 1966) and  $\alpha$ -methyl-noradrenaline (Boakes et al., 1971); whereas the depressant responses could be antagonised by mescaline (Gonzalez-Vegas, 1971), 3,4-dimethoxyphenylethylamine (Gonzalez-Vegas & Wolstencroft, 1971a), and bulbocapnine (Gonzalez-Vegas & Wolstencroft, 1971b).

##### b. Superior colliculus

Straschill & Perwein (1971) described a depressant action of NA on collicular neurones.

##### c. Substantia nigra

Kemp et al. (1977) described both excitatory and depressant responses to NA on single neurones in the substantia nigra; biphasic responses also occurred.

#### d. Vestibular nuclei

In the lateral vestibular nucleus, Yamamoto (1967) described an excitatory action of NA on single neurones. This observation has been recently confirmed by Kirsten & Sharma (1976). Yamamoto (1967) found that the excitatory responses were not antagonised by phentolamine, but could be blocked by dichloroisoprenaline (DCI). On the other hand, Kirsten & Sharma (1976) found that the excitatory responses could be abolished by phentolamine, while sotalol or propranolol were without effect.

In contrast to the lateral vestibular nucleus, a depressant action of NA was described on neurones in the medial vestibular nucleus; these depressant responses could not be modified by either the  $\alpha$ -adrenoceptor blocking agent phentolamine, or the  $\beta$ -adrenoceptor blocking agents sotalol and propranolol (Kirsten & Sharma, 1976).

#### e. Raphe nuclei

Both excitatory and depressant responses to NA were described on neurones of the raphe nuclei (Couch, 1970).

#### f. Locus coeruleus

Only depressant responses to NA were reported on neurones of the locus coeruleus (Svenson et al. 1975; Cederbaum & Aghajanian, 1976). These cells were depressed by three other adrenoceptor stimulants (adrenaline, isoprenaline and clonidine); the depressant responses could be antagonised by the  $\alpha$ -adrenoceptor blocking agent piperoxane, but were not affected by the  $\beta$ -adrenoceptor blocking agent sotalol (Cederbaum & Aghajanian, 1976).

#### I.4.3. CEREBELLUM

Yamamoto (1967) observed both excitatory and depressant neuronal responses to NA in the cerebellar flocculus; approximately two-thirds of the NA-sensitive cells responded with excitation, and one-third with depression. On the other hand, NA had an almost exclusively depressant effect on neurones in the neocerebellar cortex.

Detailed studies have been made of the pharmacology of cerebellar Purkinje cells in the past few years. These cells can be identified by their characteristic firing, and, due to their large size, are eminently suitable for intracellular recording. Only a depressant action of NA has been described on cerebellar Purkinje cells (Hoffer et al., 1971b; Siggins et al., 1971a; Curtis et al., 1971; Godfraind & Pumain, 1972; Lake & Jordan, 1974; Woodward et al., 1974; Freedman & Hoffer, 1975; Kostopoulos & Yarbrough, 1975; Freedman et al., 1975; Hoffer et al., 1976; Yarbrough, 1976; Freedman et al., 1976). Experiments involving intracellular recording (Siggins et al., 1971b) showed that NA hyperpolarized the membrane of Purkinje cells; this hyperpolarization was accompanied by a decrease in membrane conductance.

The depressant responses of Purkinje cells to NA could be antagonised by the  $\beta$ -adrenoceptor blocking agent sotalol (Hoffer et al., 1971b; Woodward et al., 1974) and by the neuroleptics fluphenazine (Freedman & Hoffer, 1975; Hoffer et al., 1976) and  $\alpha$ -flupenthixol (Freedman & Hoffer, 1975). The responses to NA could be potentiated by the tricyclic antidepressant drug desipramine (Hoffer et al., 1971b).

Siggins et al. (1971a; 1971b) suggested that the depressant responses of cerebellar Purkinje cells to NA were mediated by cyclic-AMP: the depressant effects of NA were mimicked by cyclic-AMP, and the effects of both NA and cyclic-AMP were potentiated by theophylline and aminophylline, drugs which inhibit the enzyme phosphodiesterase and thus increase the concentration of cyclic-AMP in the tissue. However, Godfraind & Pumain (1972) and Lake & Jordan (1974) could not replicate the observations of Siggins et al. (1971a). These discrepant results have been attributed to technical differences between the laboratories concerned (Bloom, 1975).

#### 1.4.4. HYPOTHALAMUS

##### a. Supraoptic nucleus

Barker et al. (1971) studied the effects of NA, dopamine and acetylcholine on neurones in the supraoptic nucleus. Only depressant responses to NA occurred on supraoptic neurones; there was no difference between neurosecretory (i.e. cells responsible for the secretion of the antidiuretic hormone, ADH) and non-neurosecretory cells, in this respect. The depressant responses to NA could be antagonised by the  $\beta$ -adrenoceptor blocking agent sotalol, and could be potentiated by the tricyclic antidepressant drug desipramine. Sakai et al. (1974) also observed a depressant action of NA on supraoptic neurones in an in vitro organ culture preparation.

b. Suprachiasmatic nucleus

Neurones in the suprachiasmatic nucleus are thought to be involved with the regulation of the circadian rhythm of pineal enzyme content (Moore & Klein, 1974); these neurones are also functionally linked to the visual system (Nishino et al., 1976). Nishino & Koizumi (1977) examined the effects of NA<sup>on</sup> suprachiasmatic neurones. The cells could be divided into three groups: cells responding with an increase, a decrease, or no change in their firing frequency to optic nerve stimulation. NA could evoke both excitatory and depressant responses on neurones belonging to either of the three neuronal groups.

c. Preoptic nuclei

i. Cells activated by the stimulation of the basal hypothalamus.

It is believed that these cells are involved in the control of ovulation (Dyer, 1973). Only depressant responses to NA have been described on these neurones (Dyball & Dyer, 1974 ; Dyball et al., 1974; Whitehead & Ruf, 1974).

ii. Thermosensitive cells. Both excitatory and depressant responses to NA have been described on thermosensitive neurones in the preoptic and anterior hypothalamic area (Beckman & Eisenman, 1970; Hori & Nakayama, 1973; Jell, 1973; Murakami, 1973). No significant difference could be found between thermosensitive and thermo-insensitive cells on the basis of their responsiveness to NA (Jell, 1973). When the responsiveness to NA of warm-sensitive and cold-sensitive cells were compared, the warm-sensitive cells appeared to be usually depressed

and the cold-sensitive cells usually excited by NA (Beckman & Eisenman, 1970; Murakami, 1973). On the other hand, 5-hydroxy-tryptamine tended to excite the warm sensitive cells, and depress the cold-sensitive ones (Murakami, 1973).

#### d. Paraventricular nucleus

Neurosecretory cells in this area are thought to be responsible for the secretion of the hormone oxytocin (see Moss et al., 1972). The neurosecretory cells can be identified in this nucleus by the antidromic stimulation of the axons in the neural hypophysis (Sundsten et al., 1970). NA could evoke responses on both neurosecretory and non-neurosecretory cells: while almost all the NA-sensitive neurosecretory cells responded with depression (Cross et al., 1971; Moss et al., 1972), the predominant action of NA was excitation on the non-neurosecretory cells (Moss et al., 1972).

#### e. Tuberal nuclei

This area of the hypothalamus (arcuate nucleus and median eminence) is thought to be responsible for the production of releasing and inhibitory factors which control the secretion of gonadotrophic hormones (McCann & Porter, 1969). Geller (1976) studied the sensitivity of neurones to potential neurotransmitters in these nuclei in vitro using explant tissue cultures. NA applied by microelectrophoresis could evoke both excitatory and depressant responses; the majority of the neurones, however, were depressed by NA in this preparation.



#### I.4.5. THALAMUS

##### a. Ventrobasal nuclear complex

These thalamic nuclei contain the thalamo-cortical relay neurones which can be identified by their antidromic responses to stimulation of the somatosensory cortex. Both excitatory and depressant responses to NA could be observed on neurones in the ventrobasal thalamus; while more superficially situated neurones were usually depressed, deeper neurones responded with excitation or depression in approximately equal proportions (Phillis & Tebēcis, 1967a; 1967b).

##### b. Medial geniculate nucleus

Tebēcis (1967; 1970) observed excitatory, depressant and biphasic responses to NA on unidentified medial geniculate neurones; the majority of the NA-sensitive cells responded with depression. Repeated applications of NA lead to desensitisation of excitatory, but not of depressant responses. Strychnine blocked the depressant effects of NA, but only when applied with much higher ejecting currents than were required to antagonise depressant responses to glycine.

##### c. Lateral geniculate nucleus

Curtis & Davis (1962) and Phillis et al., (1967) found that NA depressed the orthodromic field potential and spontaneous firing of lateral geniculate neurones. On the other hand, Satinsky (1967) reported both excitatory and depressant responses to NA in the lateral geniculate nucleus.

#### I.4.6. LIMBIC STRUCTURES

##### a. Hippocampus

Most reports describe only a depressant action of NA on hippocampal neurones (Stefanis, 1964; Herz & Nacimient, 1965; Salmoiraghi & Stefanis, 1965; Biscoe & Straughan, 1966; Segal, 1974; Segal & Bloom, 1974; Segal, 1976). The depressant responses to NA could be antagonised by the  $\beta$ -adrenoceptor blocking agent sotalol, and potentiated by the tricyclic antidepressant drug desipramine (Segal & Bloom, 1974). The depressant responses could also be modified by drugs interfering with the cyclic-AMP system: the phosphodiesterase inhibitor papaverine potentiated the responses, whereas prostaglandins of the E series antagonised them, suggesting the involvement of cyclic-AMP in the mediation of the responses to NA (Segal & Bloom, 1974). There are some indications, however, that NA may also have an excitatory action on hippocampal neurones: Segal & Bloom (1974) reported that the depressant responses to NA were often followed by a 'rebound' excitation.

##### b. Septal nuclei

Only a depressant effect of NA has been reported on neurones in this brain area (Herz & Gogolak, 1965; Segal, 1974b).

### c. Olfactory bulb

Only a depressant action of NA has been described on mitral cells in this structure (Baumgarten et al., 1963; Bloom et al., 1964; Salmoiraghi et al., 1964; McLennan, 1971). The depressant responses to NA could be blocked by the  $\alpha$ -adrenoceptor blocking agent dibenamine, but not by the  $\beta$ -adrenoceptor blocking agent dichloroisoprenaline (DCI) (Salmoiraghi et al., 1964).

In contrast to the mitral cells, granule cells in the olfactory bulb were excited by NA, and this excitation was usually accompanied by a depression of the firing of the mitral cell recorded simultaneously (McLennan, 1971). McLennan (1971) suggested that the depression of mitral cells by NA might be mediated by the excitation of granule cells which have inhibitory synaptic contacts with the mitral cells.

### I.4.7. CORPUS STRIATUM

Herz & Zieglgänsberger (1968) described only depressant responses to NA on single neurones in the caudate nucleus; most other authors, however, reported both excitatory and depressant responses (Bloom et al., 1965; Salmoiraghi & Stefanis, 1965; York, 1970; Spencer & Havlicek, 1974; Bevan et al., 1975c). The excitatory responses to NA could be antagonised by the  $\alpha$ -adrenoceptor blocking agent phentolamine, whereas the depressant responses could be antagonised by the  $\beta$ -adrenoceptor blocking agents sotalol and INFEA (York, 1970). Both the excitatory and the depressant responses could be potentiated by the tricyclic antidepressant drug desipramine (Bevan et al., 1975c).

#### I.4.8. CEREBRAL CORTEX

For review of the literature see IV.1.

#### I.4.9. CONCLUSIONS

The review of the literature shows that, in most structures of the brain (spinal cord, brain stem, hypothalamus, thalamus, limbic system, corpus striatum, cerebral cortex), NA has a dual effect: both excitatory and depressant responses have been described. These observations suggest that NA can activate both excitatory and inhibitory receptors on central neurones. A study of the distribution of these receptors suggests that while some neurones may contain only one kind of receptor, other neurones may possess both excitatory and inhibitory receptors for NA. Thus neurones in the lateral vestibular nucleus (Yamamoto, 1967), or cold-sensitive cells in the anterior hypothalamus (Murakami, 1973) appear to possess only excitatory receptors, whereas cerebellar Purkinje cells (Hoffer et al., 1971b), or warm-sensitive cells in the anterior hypothalamus (Murakami, 1973) seem to have only inhibitory receptors. The occurrence of biphasic responses to NA in several structures (e.g. spinal cord, brain stem reticular formation, medial geniculate nucleus) suggests that both excitatory and inhibitory receptors may be present on the same cells (see Headley & Lodge, 1976; Hösli et al., 1971; Tebēcis, 1970).

There are also reports concerning the effects of both  $\alpha$ - and  $\beta$ -adrenoceptor blocking agents on neuronal responses to NA. Although no general conclusions can be drawn from these experiments,

some of the observations are consistent with the hypothesis that the excitatory responses may be mediated by  $\alpha$ -, and the depressant responses by  $\beta$ -adrenoceptors. Thus the excitatory responses to NA could be antagonised by  $\alpha$ -adrenoceptor blocking agents in the lateral vestibular nucleus (Kirsten & Sharma, 1976) and in the striatum (York, 1970), whereas the depressant responses could be abolished by  $\beta$ -adrenoceptor blocking agents in the cerebellum (Hoffer *et al.*, 1971b), supraoptic nucleus (Barker *et al.*, 1971), hippocampus (Segal & Bloom, 1974) and striatum (York, 1970). On the other hand, there are also observations which suggest that depressant responses to NA may be mediated by  $\alpha$ -adrenoceptors in some structures of the brain: depressant responses to NA of neurones in the locus coeruleus (Cedarbaum & Aghajanian, 1976), and of mitral cells in the olfactory bulb (Salmoiraghi *et al.*, 1964) could be antagonised by  $\alpha$ -adrenoceptor blocking agents, but not by  $\beta$ -adrenoceptor blocking drugs. It should be noted, however, that the observations of McLennan (1971) in the olfactory bulb suggest that the depressant responses of mitral cells to NA may be mediated by excitatory NA-receptors situated on inhibitory interneurones.

#### 1.5. TRICYCLIC ANTIDEPRESSANT DRUGS AND THE ADRENERGIC SYSTEM

The term 'tricyclic antidepressant' refers to a group of compounds with a structure and pharmacological action profile which is similar to that of imipramine. Imipramine is a dibenzazepine derivative with a structural formula very similar to that

of chlorpromazine. The clinical antidepressant properties of imipramine were discovered by Kuhn in 1957; since then a large number of similar compounds (e.g. desipramine, amitriptyline, nortriptyline, protriptyline) have been synthesized. The pharmacological actions of these drugs have been studied extensively (for reviews see Domenjoz & Theobald, 1959; Klerman & Cole, 1965; Gyermek, 1966; Bickel, 1968; Bopp & Biel, 1974). Similarly to chlorpromazine, these drugs possess anticholinergic, antihistamine, antiserotonin, adrenolytic and local anaesthetic properties. In contrast to chlorpromazine, however, these drugs reverse the reserpine-induced sedation in rodents, and potentiate the behavioural and motor responses produced by amphetamine and L-DOPA. These effects have been used in the pharmacological screening for compounds with potential antidepressive properties. The most characteristic and most widely studied effect of these drugs, however, is their interaction with the adrenergic system.

#### 1.5.1. UPTAKE BLOCKADE

NA applied to sympathetically innervated tissues is accumulated into sympathetic nerve terminals by an active, temperature-dependent, energy-requiring process. It is believed that the re-uptake of the released NA by this active 'membrane pump' is the most important way of eliminating the transmitter from the synaptic gap. A similar active uptake process for NA has been described in brain tissue. The active uptake of NA into nerve terminals can be

inhibited by a variety of compounds, such as cocaine, sympathomimetic amines, phenothiazines, and tricyclic antidepressants. In fact the tricyclic antidepressants are among the most potent inhibitors of NA uptake both in the periphery and in the CNS. (For reviews see Iversen, 1967; Iversen, 1974; Iversen, 1975; see also VIII.1. and IX.1.).

#### I.5.2. POTENTIATION OF ADRENERGIC RESPONSES

The tricyclic antidepressants have been shown to be able to potentiate the responses of smooth muscle preparations to exogenously applied NA. Thus potentiation of the effects of NA has been described in the nictitating membrane (Sigg, 1959; Soffer & Gyermek, 1961; Sturman, 1971); in the vascular bed (Osborne & Sigg, 1960; Osborne, 1962); in isolated arteries (Türker & Khairallah, 1967; Bassett et al., 1969; Hrdina & Ling, 1970; Torda, 1971; McCulloch & Story, 1972); in the vas deferens (Westfall, 1973); in the anococcygeus muscle (Doggrell & Woodruff, 1977). The tricyclic antidepressants can also potentiate tissue responses to sympathetic nerve stimulation, especially when low frequencies of stimulation are used (Sigg et al., 1963; Haefely et al., 1964; Sturman, 1971; Torda, 1971).

It is generally believed that there is a close relationship between the blockade of the neuronal uptake of NA and the potentiation of adrenergic responses: uptake blockade results in a higher concentration of NA at the postsynaptic receptors, and this results in an increased response (see Iversen, 1974). This

hypothesis is supported by the results of denervation experiments: no potentiation of the effects of NA by tricyclic antidepressants could be observed in sympathetically denervated smooth muscle preparations (Gyermek & Possemato, 1960; Callingham, 1967; Sturman, 1971; McCulloch & Story, 1972; Westfall, 1973).

There are reports, however, which suggest that uptake blockade and potentiation do not necessarily co-exist in the same preparation. Thus in the Auerbach's plexus - longitudinal smooth muscle preparation of the guinea pig, tricyclic antidepressants fail to potentiate tissue responses to NA, although they effectively block the uptake of NA (Govier et al., 1969). On the other hand, the tricyclic antidepressant iprindole potentiates tissue responses to NA, although it does not block the uptake of NA (Gluckman & Baum, 1969; Lahti & Maickel, 1971).

### I.5.3. ADRENOLYTIC EFFECTS

The tricyclic antidepressants, similarly to chlorpromazine, are able to antagonise the effects of NA in smooth muscle preparations. This antagonistic effect of the antidepressants usually appears at higher concentrations than are required for the potentiation of the responses to NA. An  $\alpha$ -adrenoceptor blocking effect of these drugs has been described by Callingham (1967); Türker & Khairallah, (1967); Bassett et al. (1969); Scriabine (1969); Hrdina & Ling (1970); Torda (1971); McCulloch & Story (1972); Westfall (1973). There is less evidence, however, concerning a possible  $\beta$ -adrenoceptor blocking effect of the tricyclic antidepressants (Govier et al., 1967; Scriabine, 1969; Tamayo et al., 1973).



CHAPTER II

THE RELEASE OF NORADRENALINE FROM MICROPIPETTES

## II.1. INTRODUCTION

The time sequence of events occurring during the application of a drug by microelectrophoresis can be summarized in the following way: ejecting current pulse  $\rightarrow$  electrophoretic release of the drug from the micropipette  $\rightarrow$  concentration changes at the receptor sites  $\rightarrow$  neuronal response. The relationship between ejecting current and response cannot be interpreted unless the intervening processes are fully understood. In this chapter I shall describe experiments which examine the relationship between ejecting current pulse and the rate of release of noradrenaline from micropipettes. As a framework for discussion I shall use a theoretical model of ion movements in micropipettes.

### II.1.1. A MODEL OF ION MOVEMENTS IN MICROPIPETTES

It is proposed that the orifice at the tip of the micropipette can be regarded as a hypothetical boundary separating two fluid compartments: the drug solution in the micropipette and the external medium. Thus, drug release can be defined as the passage of drug molecules across this boundary into the external medium.

Drug release consists of spontaneous release (resulting from diffusion and hydrodynamic outflow of the solution), and electrophoretic release (resulting from iontophoresis and electro-osmosis) (Curtis, 1964). The model deals only with diffusional and iontophoretic drug release. Hydrodynamic outflow has been neglected since its contribution to spontaneous release is likely to be very small under normal experimental conditions (Curtis, 1964), especially when dilute drug solutions are used as was the case in most of our experiments. The contribution of electro-

osmosis to total electrophoretic release is reckoned to be small (Krnjević, Mitchell & Szerb, 1963b; Curtis, 1964), although it may be important in the case of poorly ionised or less soluble compounds (Curtis, 1964). In the experiments described in this chapter electrophoretic release resulting from electro-osmosis could not be differentiated from that resulting from iontophoresis, and thus, for the sake of simplicity, electrophoretic release was regarded as resulting entirely from iontophoresis.

a. Ion transport in the absence of an electrophoretic current

Let us assume that the drug solution in the micropipette is an infinitely large diffusional source, and the external medium is an infinitely large diffusional sink. Thus, after the tip of a micropipette has been immersed in the external medium at time  $t = 0$  (see Fig. 1/II), there will be a period of gradually declining rate of diffusional release followed by the establishment of a steady-state rate of release, at  $t = t_1$ . The initial period of decreasing rate of release reflects the development of an 'interphase layer' between the bulk of the drug solution in the micropipette and the external medium. The establishment of a steady-state rate of release implies the existence of an interphase layer of unchanging thickness. Pick's first law of diffusion is applicable to this condition:

$$R_D = D \frac{dc}{dx} \pi r^2 \quad (1)$$

where  $R_D$  is the rate of diffusional release,  $D$  is the diffusion coefficient,  $\frac{dc}{dx}$  is the concentration gradient across the tip boundary, and  $r$  is the radius of the tip. For the sake of simplicity, the concentration gradient may be regarded as being linear across the whole of the interphase layer.

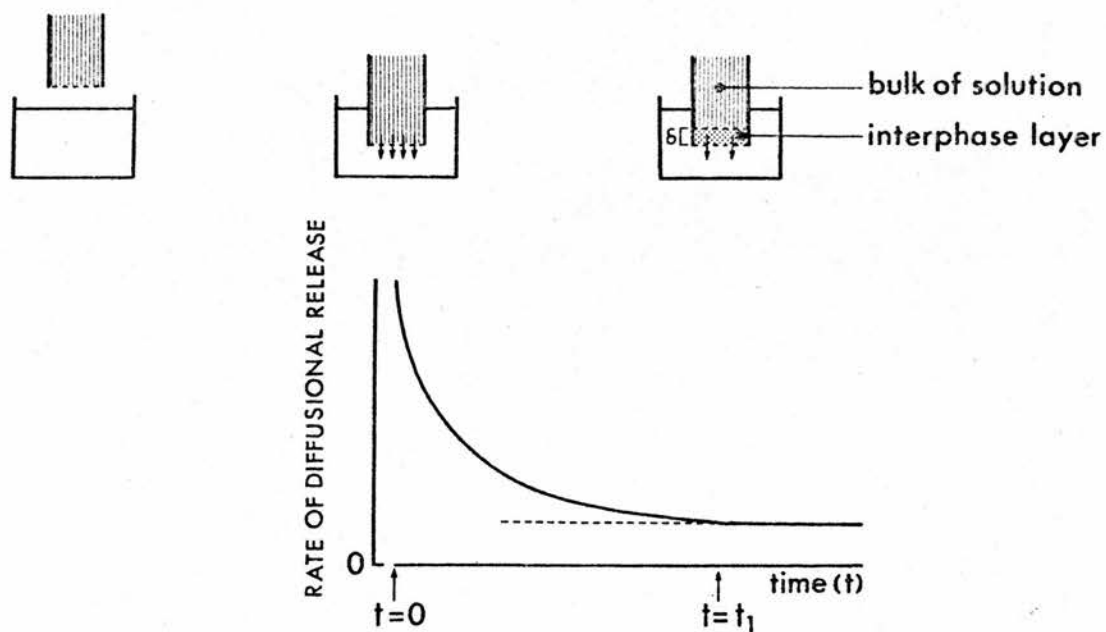


FIGURE 1/II. Hypothetical changes in the rate of diffusional release from a micropipette freshly immersed in an external medium.

The development of an interphase layer within the tip of the micropipette is shown above the graph.  $\delta$ : thickness of the interphase layer.

This assumption of linearity is also made in the derivation of the Planck-Henderson equation for the diffusional potential across an interphase region. The assumption is necessary, since without it any mathematical treatment of ion transport in the interphase layer becomes unmanageable (Bockris & Reddy, 1970; pp. 417-420). On the basis of the approximation,  $R_D$  can be expressed as

$$R_D = D \frac{c - c_o}{\delta} \pi r^2 \quad (2)$$

where  $c$  is the concentration of the drug ions in the bulk of the solution,  $c_o$  is the concentration of drug just outside the tip, and  $\delta$  is the thickness of the interphase layer. Since  $c_o \ll c$ , equation (2) can be simplified:

$$R_D = \frac{Dc \pi r^2}{\delta} \quad (3)$$

#### b. Ion transport during the passage of an electrophoretic current

Ions can be transported in a solution either under the influence of a concentration gradient (diffusion) or under the influence of an electrostatic potential gradient (iontophoresis). When both forces are acting, the net ion flux (rate of ion transport) will reflect the algebraic sum of the two forces (Bockris & Reddy, 1970). When a retaining potential is applied to the drug solution within the micropipette, these forces act in opposite directions; when an ejecting potential is applied, both forces act in the same direction.

Retaining currents. When a retaining potential is applied, ion transport in the bulk of the solution and in the interphase layer will be affected differently. In the bulk of the solution (where there is no

concentration gradient) there will be only one ion flux, the iontophoretic flux ( $R_i$ ). In the interphase layer the net ion flux ( $R_T$ ) will be the algebraic sum of the inward iontophoretic flux ( $R_i$ ) and the outward diffusional flux ( $R_D$ ):  $R_T = R_i - R_D$ . The diffusional ion transport across the tip boundary (diffusional release) in the presence of a retaining current will reflect the relationship between the ion fluxes. If  $R_i < R_D$ , there will be some diffusional release, if  $R_i > R_D$ , there will be no outward ion transport across the tip boundary.

The relationship between time and rate of release in the presence of a retaining current is shown in Figure 2/II. If a relatively weak retaining current is applied at  $t = 0$ , it will not initially counteract diffusional leakage, i.e.  $R_i < R_D$ . However, an unopposed ionic drift ( $R_i$ ) will start in the bulk of the solution instantaneously. Thus, the boundary of the bulk will drift inwards, away from the tip, and the thickness of the interphase layer will increase. Since the rate of diffusional flux in the interphase layer is inversely related to the thickness of the interphase layer (see equation (3)), the rate of diffusional release will gradually decrease, until at  $t = t_{\min}$ ,  $R_i = R_D$ . From this time on, there will be no diffusional release. Thus, a retaining current, which initially is not adequate, can, after some time ("minimal effective retention time",  $t_{\min}$ ), become effective. If the application of a retaining current is continued for longer than  $t_{\min}$ , the ionic drift in the bulk of the solution will further increase the thickness of the interphase layer, and this will further decrease the diffusional flux in the interphase layer ( $R_i > R_D$ ). Under these circumstances, the lower boundary of the interphase layer will move away from the tip

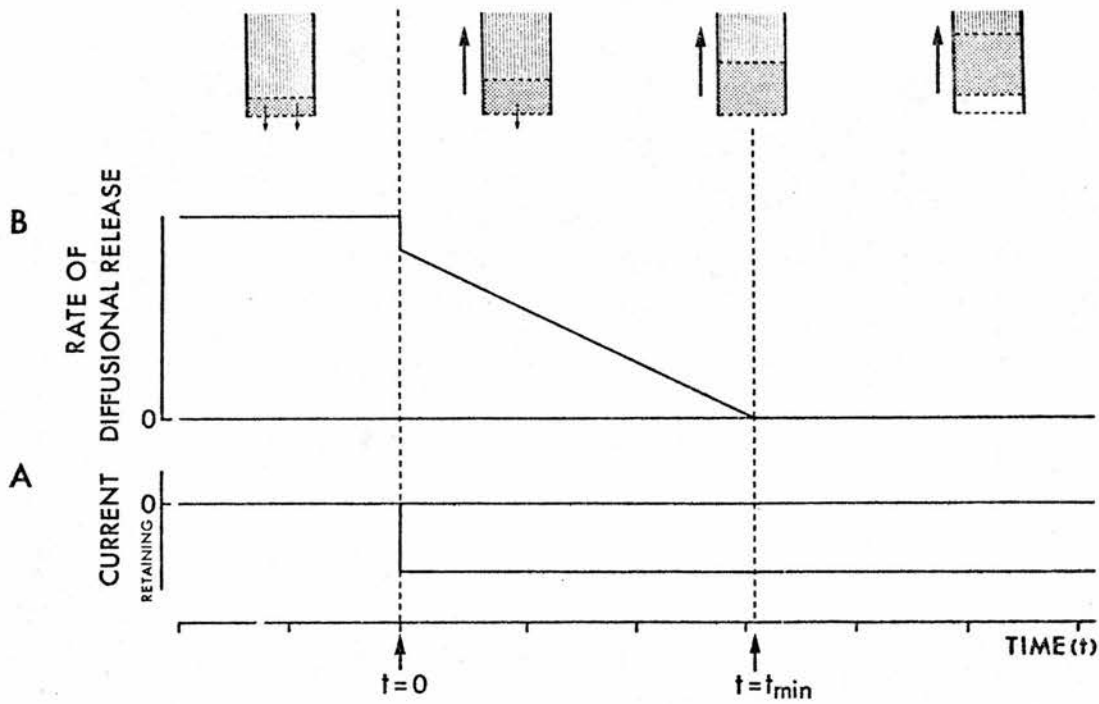


FIGURE 2/II. Hypothetical decrease in the rate of diffusional release from a micropipette in the presence of a retaining current.

A: electrophoretic current; B: rate of diffusional release. The positions of the concentration layers within the micropipette are shown at the top of the figure. In the presence of the retaining current the rate of diffusional release decreases in time; for the sake of simplicity this is shown as a linear function.

boundary, and the terminal part of the micropipette will become devoid of drug ions. Three "concentration layers" can be distinguished at this stage: the bulk of the solution, the interphase layer, and the terminal depleted layer. The thickness of these layers is determined by both parameters (intensity and duration) of the retaining current pulse. The distance travelled by the boundary of the bulk during the retention period depends upon the velocity of the ionic drift in the bulk (determined by the intensity of the retaining current) and on the retention time.

The minimal effective retention time can be calculated. Let us assume that the terminal part of the micropipette has a cylindrical shape of radius  $r$ , and the thickness of the interphase layer is  $\delta_0$  at time  $t = 0$ , when the application of the retaining current starts. After this point in time the boundary of the bulk of the solution migrates gradually away from the tip, until at  $t = t_{\min}$ , when the thickness of the interphase layer is  $\delta_c$  (critical thickness of the interphase layer),  $R_D = R_i$ . At  $t = t_{\min}$  the boundary between the bulk of the solution and the interphase layer will have moved through a distance  $\delta_c - \delta_0$ . Hence, the minimal effective retention time can be expressed as

$$t_{\min} = \frac{\delta_c - \delta_0}{v}, \quad (4)$$

where  $v$  is the drift velocity in the bulk of the solution. If, however, the bulk of the solution starts migrating from the tip boundary (as can be the case after the application of an ejecting current)  $\delta_0$  is zero. In this case, equation (4) simplifies to



$$t_{\min} = \frac{\delta_c}{v} \quad (5)$$

It is possible to derive expressions for both  $\delta_c$  and  $v$ . At  $t = t_{\min}$ ,  $R_i = R_D$ , and  $R_D = Dc \pi r^2 / \delta_c$  (Fick's first law of diffusion, see equation (3)). Therefore

$$R_i = \frac{Dc \pi r^2}{\delta_c} \quad \text{or} \quad \delta_c = \frac{Dc \pi r^2}{R_i} \quad (6)$$

The drift velocity ( $v$ ) can be expressed in terms of the iontophoretic flux ( $R_i$ ) and the concentration ( $c$ ) (Bockris & Reddy, 1970):

$$v = \frac{R_i}{c \pi r^2} \quad (7)$$

Substituting  $\delta_c$  from equation (6), and  $v$  from equation (7) equation (5) becomes:

$$t_{\min} = \frac{Dc^2 \pi^2 r^4}{R_i^2} \quad (8)$$

The iontophoretic flux ( $R_i$ ) can be obtained on the basis of Faraday's Law (see Curtis, 1964):

$$R_i = \frac{in}{zF} \quad (9)$$

where  $i$  is the intensity of the iontophoretic current,  $n$  is the transport number of the drug,  $z$  is the valency and  $F$  is Faraday's constant.

Substituting  $R_i$  from equation (9), equation (8) becomes:

$$t_{\min} = \frac{Dc_z^2 F^2 \pi^2 r^4}{i^2 n^2} \quad (10)$$

In the above derivation it was assumed that the transport number of a given ionic species is the same in the interphase layer and in the bulk of the solution. When a (negative) retaining current is applied, positive ions from <sup>the</sup> external medium (mainly sodium ions) will migrate into the fluid compartment within the micropipette. In the presence of these other ions, the transport number of the drug ions will depend upon the relative concentrations of all ionic species present. Any reduction in the transport number of the drug ions due to the presence of other ions will be greater in the interphase layer than in the bulk of the solution, because of the lower concentration of drug ions within the interphase layer. Thus a more precise version of equation (10) is

$$t_{\min} = \frac{Dc_z^2 F^2 \pi^2 r^4}{i^2 n_1 n_2} \quad (11)$$

where  $n_1$  is the transport number of the drug ions in the bulk of the solution, and  $n_2$  is the average transport number of the drug ions in the interphase layer. Since  $n_2 < n_1$ , equation (11) yields a greater value for  $t_{\min}$  than equation (10).

In deriving equation (10), it was assumed that  $r$  was constant in the terminal part of the micropipette. In reality, however, the terminal part usually has a conical shape, and thus  $r$  increases

gradually with the distance from the tip boundary. This would prolong  $t_{\min}$ , partly because the drift velocity of the boundary of the bulk will gradually decrease as the boundary migrates away from the tip, and partly because there will be a higher diffusional efflux from the bulk as the surface area of this boundary gradually increases.

$t_{\min}$  is an expression of the efficacy of a retaining current of given intensity.  $t_{\min}$  increases linearly with the square of the equivalent concentration (c.z) of the drug solution.  $t_{\min}$  also increases linearly with the diffusion coefficient of the drug ions, but it decreases with the transport number. As both the diffusion coefficient and the transport number depend upon the absolute mobility of the ions, it is apparent that a given retaining current will be more effective in counteracting the diffusional efflux of more mobile ions. Since  $t_{\min}$  increases linearly with the fourth power of the radius, it is obvious that a small increase in the size of the tip orifice will greatly reduce the effectiveness of a given retaining current.

The above theoretical considerations concerned retaining currents which were not adequate to counteract diffusional release at the beginning of their application. As retaining currents of increasing intensities are tested,  $t_{\min}$  will be gradually reduced until, in the presence of a certain retaining current ("critical retaining current")  $t_{\min} = 0$ . This retaining current (and higher retaining currents) will completely counteract spontaneous leakage at time  $t = 0$ .

As  $t_{\min}$  reflects the distance through which the boundary of the bulk of the solution has to migrate before a particular retaining current becomes effective,  $t_{\min}$  will be longer after an ejection period than after a period of steady-state diffusional release.

Ejecting currents. When an ejecting potential is applied, drug ions in the bulk of the solution drift at a steady rate towards the tip. The rate of iontophoretic transport across the tip boundary (i.e. rate of iontophoretic release), however, will change in time until the boundary of the bulk arrives at the tip boundary. The rate of release at any time is the sum of the iontophoretic and diffusional fluxes across the tip boundary:  $R_T = R_i + R_D$ .

### II.1.2. THE RELEASE CURVE

The release curve describes how the rate of release changes in time in response to an ejecting current pulse. The total release curve is obtained by the summation of the iontophoretic and diffusional curves (Fig. 3/II).

#### a. Iontophoretic release curve

The hypothetical shape of the iontophoretic release curve is shown in Fig. 3/IIB). When an ejecting potential is applied after a prior retaining current (a), the current flow starts immediately, but drug release will not start until the interphase layer reaches the tip boundary (b). There will be a further delay before the bulk of the solution reaches the tip boundary and thus a steady-state rate of release is established (c). The rate of drug release continues at this steady level until the end of the ejection period. The height of the plateau of the iontophoretic release curve is entirely determined by the intensity of the ejecting current and the transport number of the drug ions (Faraday's Law). When the application of the ejecting current is terminated (d), iontophoretic drug release stops instantaneously.

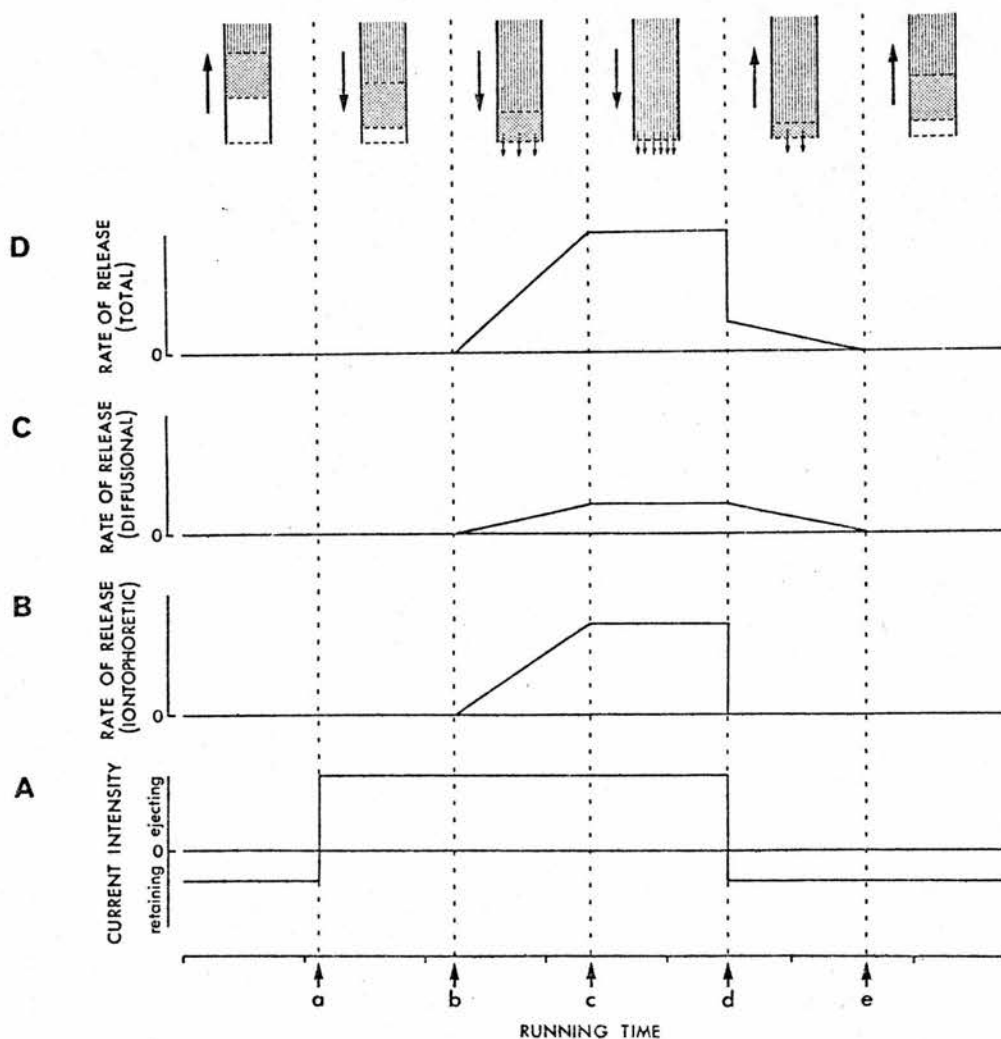


FIGURE 3/II. Hypothetical relationship between ejecting current pulse (A); iontophoretic release curve (B); diffusional release curve (C); and total release curve (D).

The positions of the concentration layers are indicated at the top of the figure. a: onset of ejecting current pulse; b: onset of drug release; c: establishment of steady-state rate of release; d: termination of ejecting pulse; e: offset of diffusional release. For the sake of simplicity, changes in the rate of release are shown as linear functions of time.

The gradual rise in the rate of release between (b) and (c) reflects a gradual increase in the transport number of drug ions in the interphase layer. (This gradual increase in transport number is due to the gradual increase in concentration of drug molecules in the interphase layer towards the bulk of the solution, since the transport number of any ion depends upon the concentration if several ionic species are present. This is inevitably the case in the tip of a micropipette, which freely communicates with the external medium.)

Since the shape of the release curve reflects the position and thickness of the "concentration layers" in the terminal part of the micropipette, it can be predicted that a higher retaining current or a longer retention time will increase the release latency (a-b) and prolong the rising phase of the release curve (b-c).

#### b. Diffusional release curve

The hypothetical (simplified) shape of the diffusional release curve is shown in Fig. 3/IIC. Since the rate of diffusional release depends on the concentration gradient across the tip boundary, the diffusional release curve follows the iontophoretic release curve. After a release latency the rate of diffusional release increases until a steady relationship is established between the two compartments during the steady-state phase of iontophoretic release. When the application of the ejecting current is terminated, diffusional release will not stop instantaneously, but will continue at a gradually decreasing rate, until the post-ejection retaining current becomes effective (Fig. 3/IIe).

### II.1.3. MEASUREMENT OF THE RELEASE OF NORADRENALINE

We have examined the shape of the release curve (rate of release plotted against time) by measuring the release of [ $^{14}\text{C}$ ]-noradrenaline (NA). There have been previous reports concerning the release of NA from micropipettes, in which either a fluorimetric assay of NA (Krnjević, Laverty & Sharman, 1963a) or, more recently the liquid scintillation technique to assay [ $^3\text{H}$ ]-NA were used (Bradley & Candy, 1970; Hoffer, Neff & Siggins, 1971). The latter technique is more sensitive but less specific than the fluorimetric method since it has been reported that as much as 50% of the radioactivity in samples of [ $^3\text{H}$ ]-NA may not be carried by NA molecules (Offerman & Merrills, 1968). Therefore, [ $^{14}\text{C}$ ]-NA was used in the present experiments.

### II.2. METHODS

Five barrelled micropipettes were constructed from Pyrex glass tubing of external diameter 1.5 mm and internal diameter 1.0 mm (Herz, Wickelmaier & Nacimientto, 1965), and were filled by boiling in distilled water under reduced pressure. The water in the barrels was replaced by the appropriate solutions by means of a thin Portex catheter. After filling, the pipettes were stored in the dark at 4°C in an atmosphere of nitrogen for at least 36 h before use. Immediately before use, the tip of the micropipette was carefully broken in order to obtain an overall tip diameter of 4.0-6.5  $\mu\text{m}$ .

Four barrels of each micropipette were filled with radioactive noradrenaline (NA) solution (0.02 M, specific activity 5 mCi/mmmole). In

some preliminary experiments in which [ $^3\text{H}$ ] -NA was used, it was found that more radioactivity was released during the passage of retaining currents than when no current was passed. Subsequent chromatographic analysis of the samples collected during the passage of retaining currents showed that the radioactivity released was not associated with NA (see also Offerman & Merrills, 1968). Therefore, because of its greater radiochemical stability, we decided to use [ $^{14}\text{C}$ ] -NA rather than the [ $^3\text{H}$ ]-labelled compound in our experiments. The lower specific activity of the [ $^{14}\text{C}$ ]-NA, however, made it necessary to dilute the radioactivity as little as possible with unlabelled NA when preparing the final solution. Accordingly, we used a 0.02 M solution in most of these experiments. In order to increase the resolution of the method when very small outputs were measured, as in the presence of retaining currents, four barrels of each micropipette were filled with the radioactive NA solution and the electrophoretic currents were applied to all of them simultaneously. Errors were further reduced by the use of long sampling periods (4-128 minutes).

Freeze-dried ( $^+$ )-noradrenaline [carbinol- $^{14}\text{C}$ ] ( $^+$ )-bitartrate (specific activity 22, 52 and 54 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England. A solution of ( $^+$ )-noradrenaline bitartrate was prepared by mixing the required amount of radioactive and non-radioactive NA to give the final solution to be used in the micropipettes (0.02 M, specific activity 5 mCi/mmol). The pH of this solution was 3.0.



An additional series of experiments (see II.3.4. and II.3.5.) was conducted using 0.2 M NA. In these experiments it was intended to examine the effect of the addition of other ionic species to the drug solution. Six-barrelled pipettes were used. Three of the barrels contained D,L-noradrenaline [carbinol-<sup>14</sup>C] (+)-bitartrate (0.2 M, pH 3.5, specific activity 0.5 mCi/mmole). The other three barrels contained a similar solution whose pH had been adjusted to 5.0 by the addition of 1 N NaOH.

The microelectrophoretic circuit used in these experiments was described by Roberts & Straughan (1967). The electrophoretic current was continuously monitored with a Pye Scalamp Galvanometer (see also III.2.4.).

The micropipette was held vertically in a micromanipulator and the tip was lowered into a small glass vial containing 1.0 ml 0.165 M NaCl solution. A silver wire, immersed into the saline solution, served as an earth electrode. Electrophoretic currents of identical intensities were applied to each of the four NA-containing barrels. Since the NA ion is a cation, positive currents were used to eject and negative currents to retain the drug ions. (Values of current intensity will always refer to the intensity of the electrophoretic current applied to each of the NA-containing barrels). The outer surface of the micropipette was washed with distilled water before the collection of each sample.

At the end of each period of collection, the contents of the vial were transferred into a glass scintillation vial. The collecting vial was washed repeatedly with the scintillator and the washings were added to the contents of the scintillation vial. A total of 10 ml of scintillator was added to each sample. The scintillator contained 0.267% PPO (2,5-diphenoxyl-oxazole), 0.0067% POPOP (1,2-bis-(5-phenyloxazol-2-yl)-benzene) in toluene with 33% Triton X-100.

Disintegrations for each sample were counted for 10 min in a Packard Tricarb liquid scintillation spectrometer. Disintegrations per minute (d/min) values were determined for each sample (after subtraction of the background), and these were converted into pmole values on the basis of the specific activity of the NA solution contained in the micropipette. Background counts per min were approximately equivalent to 2.5-3.0 pmole NA.

### II.3. RESULTS

#### II.3.1. RELEASE IN THE ABSENCE OF AN ELECTROPHORETIC CURRENT

In fourteen micropipettes (Nos. 1-14, see Table 1/II) the spontaneous release of  $[^{14}\text{C}]\text{-NA}$  was measured before any electrophoretic current had been applied. Before the first sample was collected, the tip of the micropipette was broken in order to obtain the desired tip diameter (4.0-6.5  $\mu\text{m}$ ). Then, samples were collected during successively increasing periods of time (8-128 minute). With most micropipettes, the rate of spontaneous release fell from an initially high level, until a steady-state rate of release was attained 8-64 min after the start of the experiment (Fig. 4/II).

The mean rate of spontaneous release from 14 micropipettes measured under steady state conditions was  $0.36 \pm 0.06$  pmole/barrel/min (see Table 1/II).

# RATE OF RELEASE OF NA

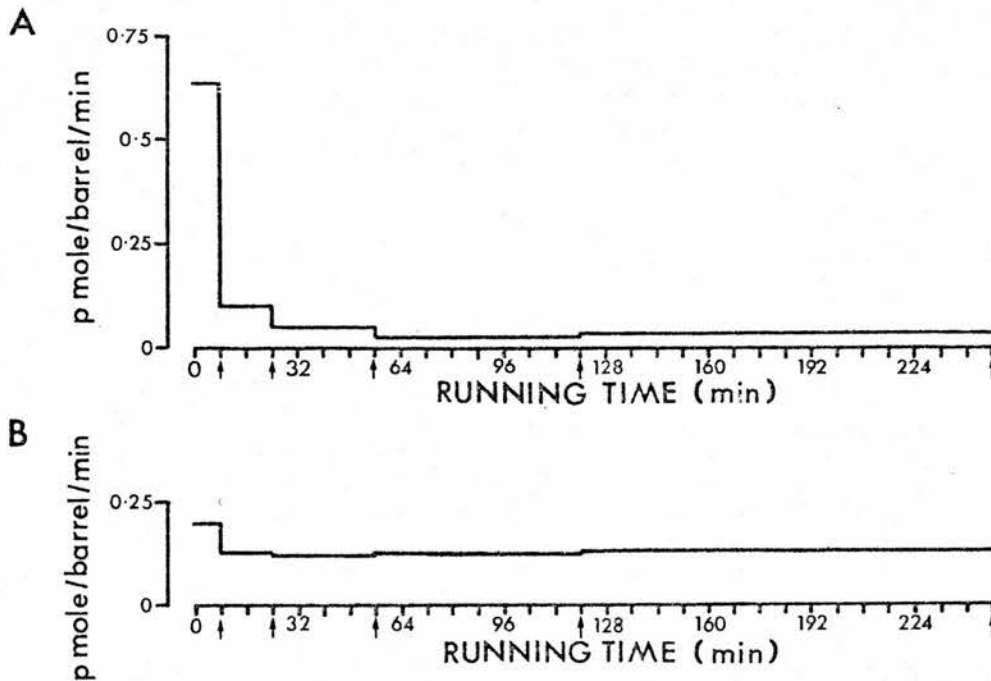


FIGURE 4/II. The rate of spontaneous release of noradrenaline from two micropipettes.

Ordinates: rate of release of noradrenaline (NA) (pmole/barrel/min).  
Abscissae: running time (min). The times at which samples were collected are indicated by arrows below the time base.

The rate of spontaneous release, measured before any current had been applied to the micropipette, declined from an initially high level until a steady-state rate was attained. A: micropipette no. 9; B: micropipette no. 4 (see Table 1/II).

### II.3.2. RELEASE IN THE PRESENCE OF A RETAINING CURRENT

#### a. When the passage of a retaining current is not preceded by an ejecting pulse

The effectiveness of various retaining currents in counteracting the spontaneous release of  $[^{14}\text{C}]$  -NA was examined with two micropipettes (nos. 10 and 14). The spontaneous output of NA during a 16 min period, during which no current was applied, was compared with the output measured during a 16 min period when a retaining current was passed. The retaining currents used were -5, -10, -15, -20 and -25 nA. Each passage of a retaining current was preceded by two successive 16 min periods of spontaneous release. From both micropipettes, a retaining current of -5 nA reduced the output to about 25% of the spontaneous release. A further reduction in the spontaneous release was observed when higher retaining currents were used; no spontaneous release could be detected when -25 nA was applied. The results obtained from micropipette No. 10 are displayed in Fig. 5/II.

#### b. When the passage of a retaining current is preceded by an ejecting pulse

A higher output was measured during the passage of a retaining current if the retention period was preceded by an ejecting pulse. The results obtained from micropipette No. 10 are shown in Fig. 5/II. Similar observations were made with micropipettes Nos. 8 and 9, with which retaining currents of up to -100 nA were tested. The output decreased with higher currents but could not be eliminated completely within the first collection period, even when -100 nA was used.

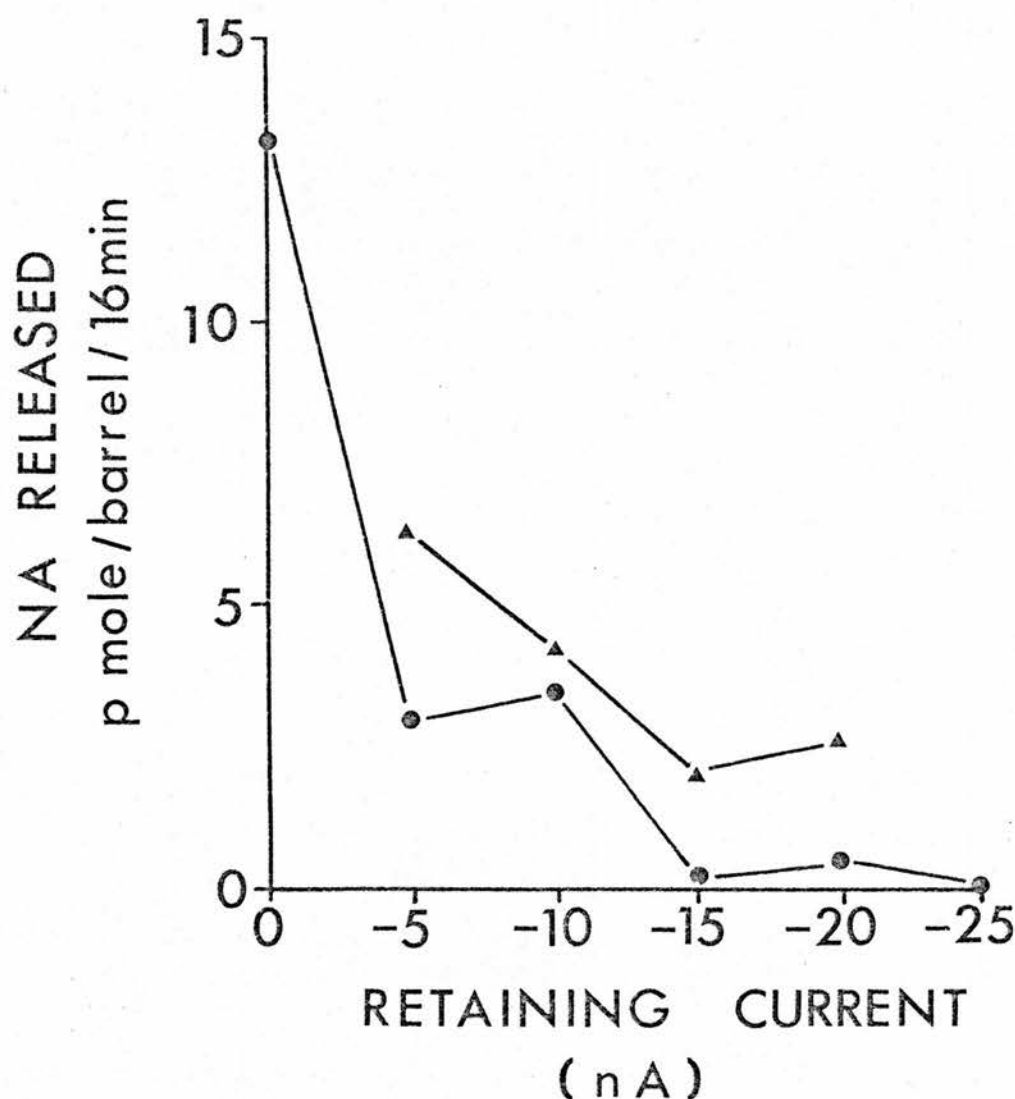


FIGURE 5/II. The relationship between the intensity of the retaining current and the release of noradrenaline from one micropipette.

Micropipette no. 10 (see Table 1/II). Ordinate: amount of noradrenaline (NA) released during each 16 min period (pmole/barrel). Abscissa: intensity of retaining current applied to each of the four NA-containing barrels (nA). Closed circles: release in the presence of a retaining current following a period (32 min) when no current was applied. Closed triangles: release in the presence of a retaining current following an ejecting pulse (+100 nA, 16 min).

The release of NA declined as higher retaining currents were passed. A retaining current was less effective following an ejecting pulse.

The time-course of the action of retaining currents was measured by passing a retaining current of given intensity continuously and measuring the output at regular intervals of 5 minutes. Every retaining current tested (-5 to -100 nA) invariably became effective if it was passed for a long enough time. This was true even of weak retaining currents (Fig. 8/IIC).

### II.3.3. RELEASE IN THE PRESENCE OF AN EJECTING CURRENT

#### a. When the passage of an ejecting current is not preceded by a retaining pulse

The electrophoretic release of [ $^{14}\text{C}$ ] -NA was measured with a wide range of ejecting current intensities (+25 to +200 nA) and a wide range of collection times (4-128 minutes). Since the prior application of a retaining current can influence subsequent drug release (see below), it was important to ensure that measurements of the rate of electrophoretic release were not subject to this distortion. Therefore retention periods or periods of spontaneous release were not interspersed between ejection periods.

In all the micropipettes tested (Nos. 1-13) the rate of release of NA was linearly related to the intensity of the ejecting current (see Fig. 6/II). The transport number of NA was calculated individually for each sample collected from each micropipette, by substitution in the following equation (Curtis, 1964):

$$n = \frac{R_1 zF}{i}$$

where  $n$  is the transport number,  $z$  is the valency (in the case of NA,  $z = 1$ ),  $F$  (coulombs) is Faraday's constant,  $i$  (amps) is the total ejecting

# RATE OF RELEASE OF NA

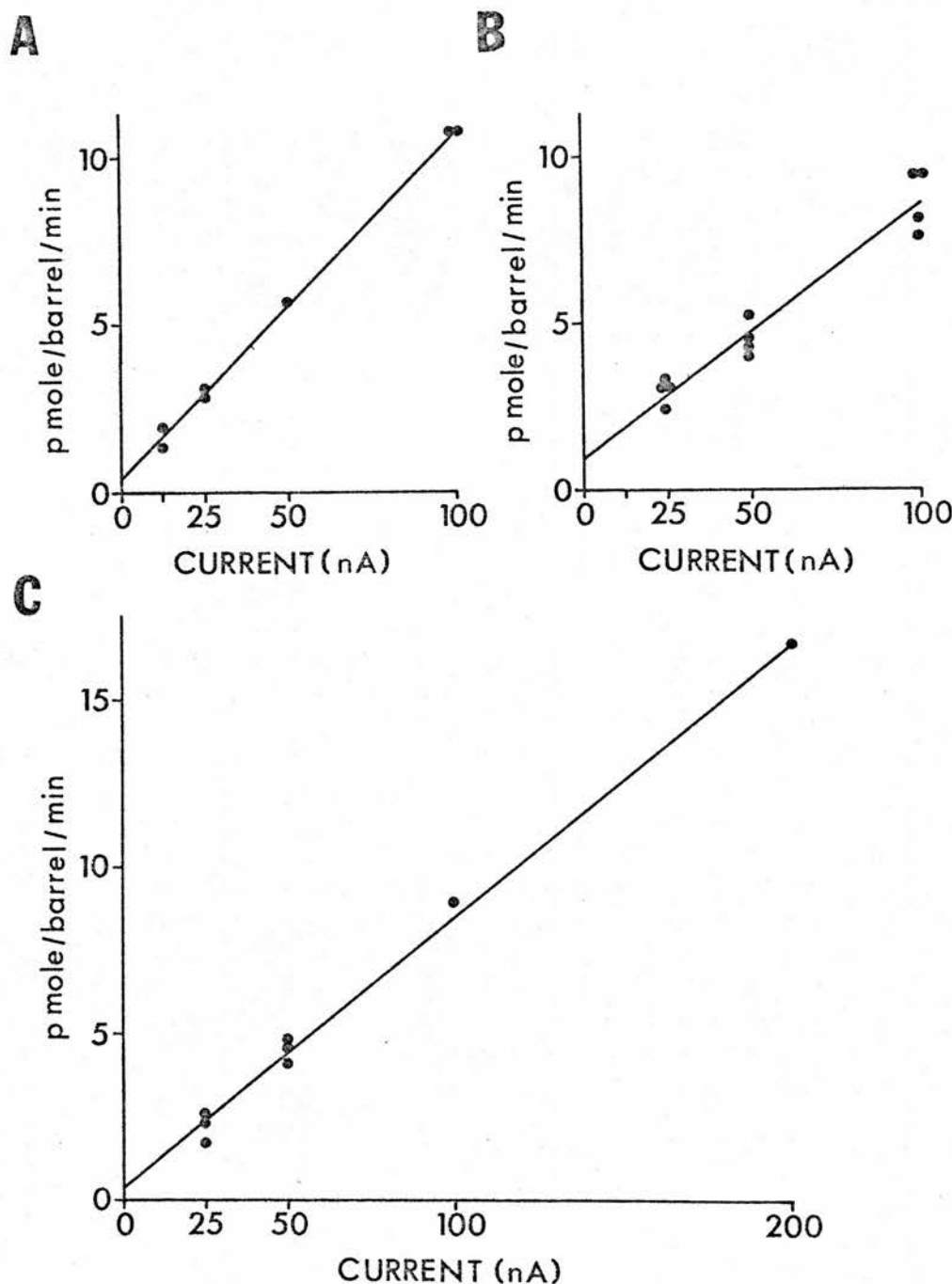


FIGURE 6/II. The relationship between the intensity of the ejecting current and the rate of release of noradrenaline from three micropipettes.

Ordinates: rate of release of noradrenaline (NA) (pmole/barrel/min).  
 Abscissae: intensity of ejecting currents applied to each of the four NA-containing barrels (nA).

The rate of release was linearly related to current intensity over a wide range of ejecting current intensities (+12.5 to +200 nA). A: micropipette no. 10 (see Table 1/II); calculated regression line:  $y = 1.623 + 0.414x$ . B: micropipette no. 7; calculated regression line:  $y = 3.690 + 0.308x$ . C: micropipette no. 2; calculated regression line:  $y = 0.832 + 0.331x$ .

TABLE 1/II. Rate of steady-state spontaneous release and transport number of NA obtained from each of the micropipettes (1-14).

Transport numbers were calculated individually for each of the samples collected from each micropipette. Four barrels of each micropipette contained [ $^{14}\text{C}$ ]-NA (0.02 M, specific activity 5m Ci/mmole).

Micro- pipette number	Tip diameter ( $\mu$ )	Rate of spontaneous release (p mole/barrel/min)	Transport number (mean $\pm$ standard deviation)
1	5	0.09	0.11 $\pm$ 0.04
2	5	0.34	0.14 $\pm$ 0.02
3	5	0.35	0.03 $\pm$ 0.00
4	4	0.12	0.19 $\pm$ 0.01
5	5	0.16	0.11 $\pm$ 0.03
6	5	0.22	0.14 $\pm$ 0.04
7	5	0.75	0.13 $\pm$ 0.02
8	5	0.32	0.29 $\pm$ 0.06
9	5	0.04	0.19 $\pm$ 0.02
10	6.5	0.82	0.17 $\pm$ 0.00
11	5	0.20	0.22 $\pm$ 0.02
12	6	0.48	0.33 $\pm$ 0.05
13	5.5	0.64	0.18 $\pm$ 0.01
14	6	0.51	not measured

The mean value of the transport number obtained from 13 micropipettes was 0.17  $\pm$  0.02.



current applied to all four barrels and  $R_i$  (mole/s) is the rate of electrophoretic release of NA (total rate of release minus steady-state rate of spontaneous release). Mean values of the transport numbers obtained from each of the micropipettes are shown in Table 1/II; the average value for the 13 micropipettes was  $0.17 \pm 0.02$ . It can be seen that the transport number of NA measured in most of the micropipettes was between 0.1 and 0.25. No correlation could be found between the transport number measured with different micropipettes and tip diameter, electrical resistance or steady-state rate of spontaneous release (product moment correlation test).

b. When the passage of an ejecting current is preceded by a retaining pulse

In microelectrophoresis experiments, the passage of an ejecting current is normally preceded by the passage of a retaining current. We, therefore, examined the release of NA during ejecting periods following retaining pulses of various intensities and durations using five micropipettes (8, 9, 12, 13 and 14).

When standard ejecting pulses were used, an increase in the intensity of the retaining current reduced the amount of NA released during the subsequent ejecting pulses. Restoration of the original retaining current resulted in a progressive increase in the output of NA over the course of the next few ejecting pulses (Fig. 7/II).

We examined whether the reduction in output brought about by a prior retaining current was due to a change in the time-course of drug release. In these experiments, the ejecting current was passed for a prolonged period and samples were collected at regular intervals of 5 min (Fig. 8/II). When no retaining current had been passed previously, the

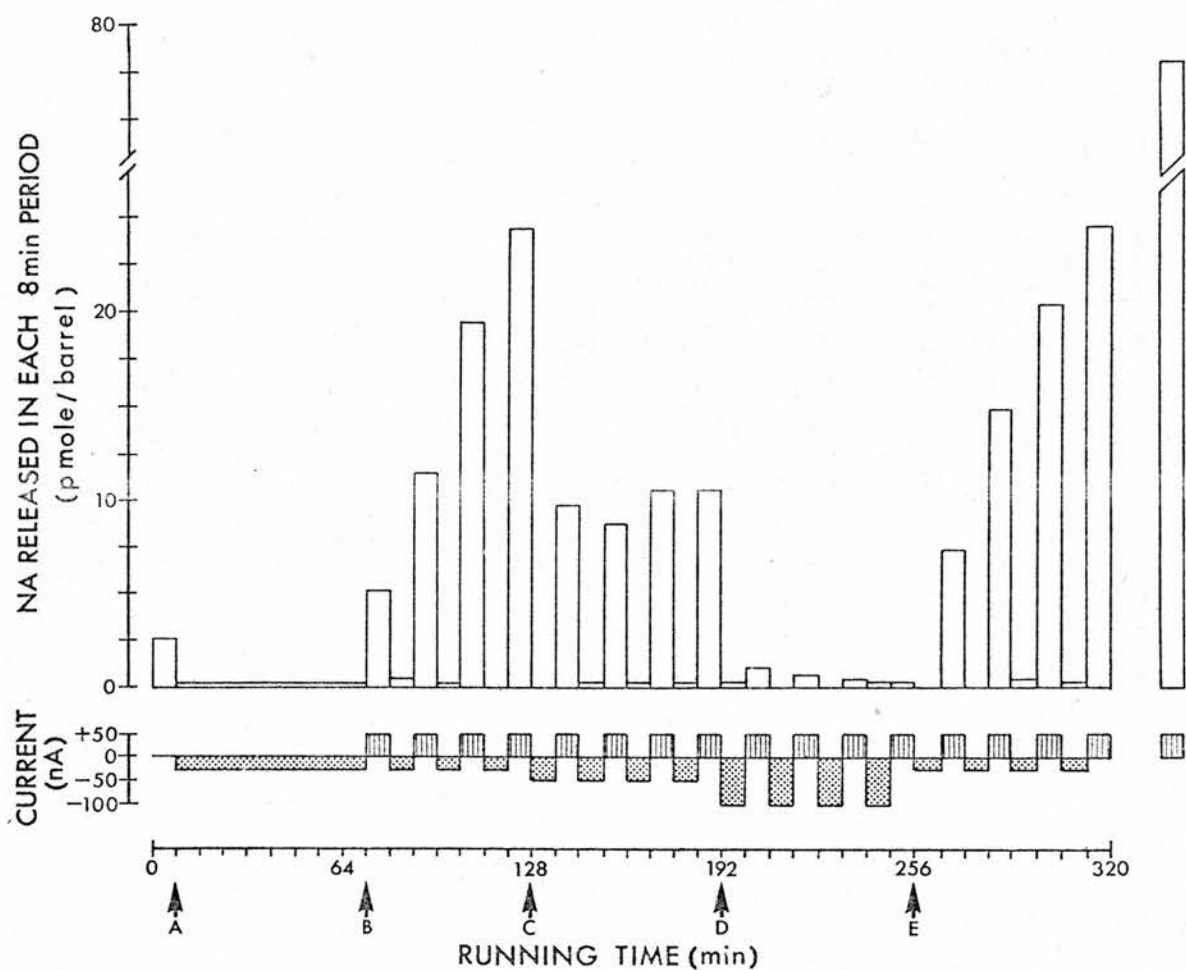


FIGURE 7/II. The effect of various retaining currents upon the electrophoretic release of noradrenaline from one micropipette.

Micropipette no. 8 (see Table I/II). Lower graph: electrophoretic current applied to each of the four noradrenaline (NA) containing barrels (ordinate: current intensity, nA, positive upwards; abscissa: running time, min). Stippled area: retaining current; hatched area: ejecting current. Upper graph: release of NA during each of the 8 min period of sample collection (ordinate: release of NA, pmole/barrel/min; abscissa: running time, the same scale as in the lower graph).

Ejecting and retaining currents were applied alternately; the parameters (intensity and duration) of the ejecting pulse were kept constant throughout the study. Changes in the parameters of the applied currents are indicated by capital letters under the time base.

After a prolonged application of a retaining current (A - B), successive ejecting pulses (B - C) evoked progressively greater outputs. Increases in the intensity of the retaining current reduced the amount of NA released by the ejecting pulse (C - D, D - E). Restoration of the original retaining pulse (E) was followed by progressively increasing outputs.

# RATE OF RELEASE OF NA

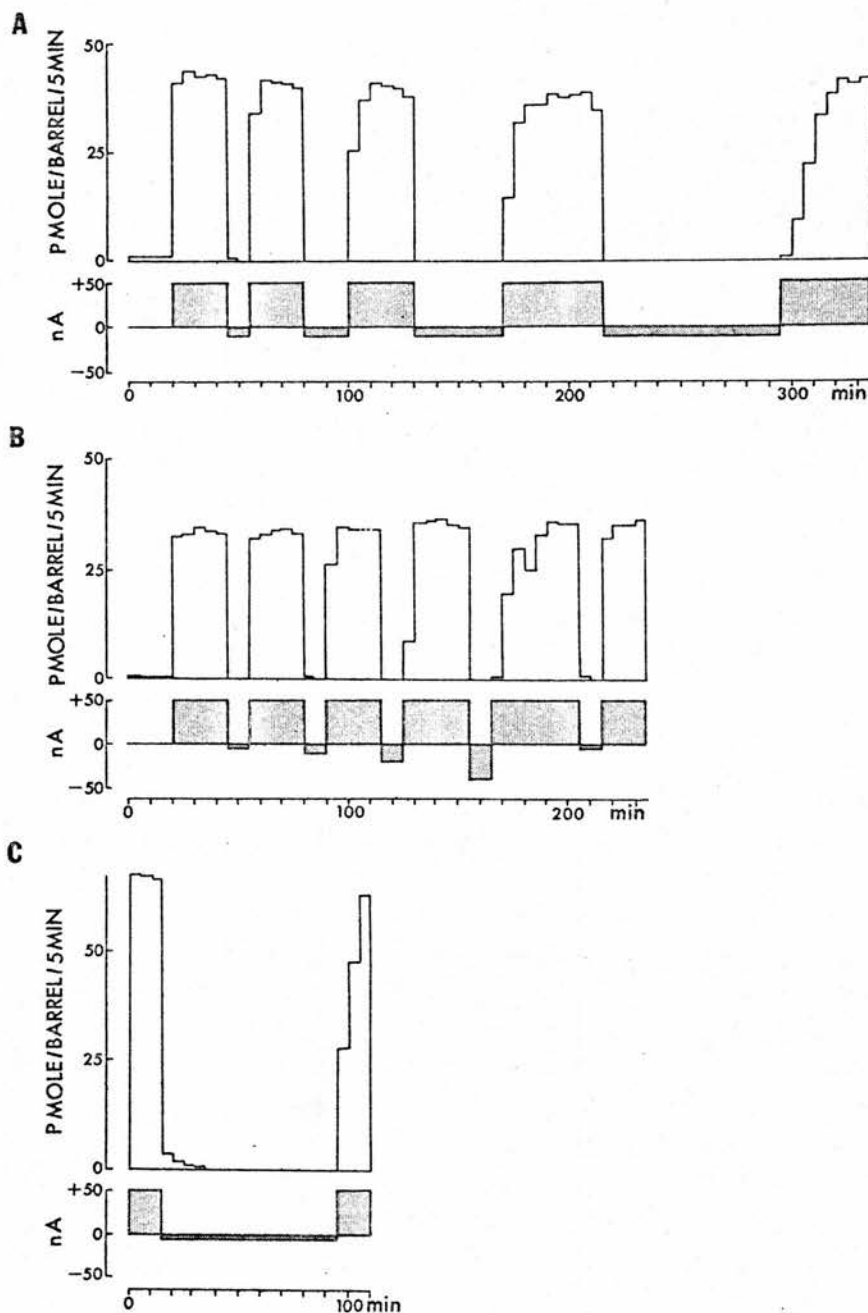


FIGURE 8/II. The effects of retaining currents on the kinetics of the electrophoretic release of noradrenaline.

Lower graph: electrophoretic currents applied (see Fig. 7/II). Upper graph: release curves for noradrenaline (NA) (ordinate: rate of release of NA, pmole/barrel/ 5 min; abscissa: running time; abscissa: running time, min).

A: micropipette no. 11 (see Table 1/II): increases in the retention time prolonged the rising phase of the release curve. B: micropipette no. 13: increases in the intensity of the retaining current prolonged the rising phase of the release curve. C: micropipette no. 12: a weak retaining current (-5 nA) did not abolish spontaneous release immediately, but prolonged the rising phase of a subsequent release curve.



passage of the ejecting current resulted in a rectangular release curve. The effect of the prior passage of a retaining current was to prolong the rising phase of the release curve without affecting the plateau rate of release. Increases in either the intensity (Fig. 8/IIB) or the duration (Fig. 8/II.A) of the retaining current magnified this distortion of the release curve. It can be seen that, after a high retaining current or a long retention time, the rate of release during the ejection period gradually increased before a steady-state rate of release was achieved. Even weak retaining currents, when passed for prolonged periods, distorted the rising phase of the release curve. In the experiment shown in Fig. 8/II.C a retaining current of -5 nA was not effective for the initial 20 min of its passage, but, after it had been passed for a further 60 min, it caused a marked distortion of the release curve during the next ejection period.

#### II.3.4. EFFECT OF CONCENTRATION

Since in our in vivo experiments more concentrated (0.05 M and 0.2 M) NA solutions were used, it was of interest to study the release of NA from micropipettes containing a 0.2 M solution. Five six-barrelled micropipettes were used in these experiments (nos. 15-19; see Table 2/II). Three barrels of the micropipettes contained [ $^{14}\text{C}$ ] -NA (0.2 M; pH 3.5; specific activity 0.5 mCi/mmol). The remaining three barrels contained a [ $^{14}\text{C}$ ] -NA solution whose pH had been adjusted by the addition of NaOH. (These latter three barrels were used to evaluate the effects of the addition of other ions; see II.3.5.) When the release from barrels containing one solution was being studied, spontaneous release from the other three barrels was either taken into account as a constant factor

to be subtracted from the total release measured, or was eliminated by the passage of a high retaining current ( $-300$  nA) which had proved instantaneously effective. Ten minute sample collection periods were used.

a. Spontaneous release

There was a considerable variation between micropipettes with respect to the rate of spontaneous release (see Table 2/II). The mean rate of release of NA from the solution at pH 3.5 (i.e. when no other ions had been added) was  $8.8 \pm 4.0$  pmole/barrel/min. Micropipette no. 15, which had a tip diameter of  $8 \mu$ , showed a very high rate of release ( $24.1$  pmole/barrel/min). If this micropipette is excluded, the mean rate of spontaneous release becomes  $4.9 \pm 1.4$  pmoles/barrel/min. This value is somewhat greater than ten times the mean rate of spontaneous release from the  $0.02$  M solution ( $0.36 \pm 0.06$  pmole/barrel/min; see II.3.1.).

There was a significant correlation between the tip diameter and the rate of spontaneous release (product moment correlation test:  $r = 0.995$ ,  $p < 0.05$ ).

b. Release in the presence of a retaining current

The efficacy of retaining currents was evaluated using two micropipettes (15 and 19). First an ejecting current ( $+100$  nA) was passed and the output during a 10 min retention period was measured. The release curve for  $+100$  nA was then repeated, and the retaining current of  $-25$  nA was applied again, this time for 20 min. The output during the 10 min retention period was subtracted from the output during the 20 min retention period in order to obtain an estimate during the

TABLE 2/II. Characteristics of micropipettes (15-19).

Transport numbers were calculated individually for each of the samples collected from each micropipette. Three barrels of each micropipette contained  $[C^{14}]$ -NA (0.2 M, pH 3.5, specific activity 0.5 mCi/mole); the other three barrels contained  $[^{14}C]$ -NA (0.2 M, pH adjusted to 5.0 with NaOH, specific activity 0.5 mCi/mole).

MICROPIPETTE NUMBER	TIP DIAMETER ( $\mu$ )	ELECTRICAL RESISTANCE OF EACH DRUG BARREL (M $\Omega$ )						RATE OF SPONTANEOUS RELEASE (pmole/barrel/ min; mean $\pm$ S.E.)			TRANSPORT NUMBER (mean $\pm$ S.E.)		
		NA (pH 3.5)		NA (pH 5.0)		NA (pH 5.0)		NA (pH 3.5)	NA (pH 5.0)	NA (pH 3.5)	NA (pH 5.0)		
		1	2	3	4	5	6						
15	8	60	60	60	25	25	25	24.1 $\pm$ 0.7	24.1 $\pm$ 0.7	0.18 $\pm$ 0.01	0.02 $\pm$ 0.00		
16	4	75	75	100	50	50	50	2.7 $\pm$ 0.7	2.0 $\pm$ 0.7	0.21 $\pm$ 0.01	0.10 $\pm$ 0.00		
17	4	65	65	65	30	30	30	3.4 $\pm$ 0.1	3.4 $\pm$ 0.1	0.28 $\pm$ 0.01	0.11 $\pm$ 0.00		
18	4	95	95	95	60	60	60	4.6 $\pm$ 0.1	4.6 $\pm$ 0.1	0.36 $\pm$ 0.01	0.18 $\pm$ 0.01		
19	5	85	85	85	40	40	40	9.1 $\pm$ 0.5	11.1 $\pm$ 0.4	0.43 $\pm$ 0.01	0.13 $\pm$ 0.01		

The mean values of the transport number obtained from 5 micropipettes were 0.29  $\pm$  0.05 (pH 3.5 solution) and 0.11  $\pm$  0.03 (pH 5.0 solution).

final 10 min of the 20 min retention period. This procedure was repeated, using successively increasing retention times up to 60 min.

It was found that the output of NA decreased in time during the prolonged application of a retaining current. The results obtained from micropipette no. 19 are illustrated in Fig. 9/II. Similar results were obtained with micropipette no. 17.

During the first 30 min retention with  $-25$  nA the amount of NA released from the  $0.2$  M solution was 72% (micropipette no. 17) and 44% (micropipette no. 19) of the amount released during an equivalent period when no current was applied. Comparison of Fig. 9/II with Fig. 8/II shows that this retaining current was much less effective in counteracting spontaneous release from the  $0.2$  M solution than were similar retaining currents in the case of the  $0.02$  M solution. With the  $0.2$  M solution, the minimal effective retention time for  $-25$  nA was more than 50 min (micropipette no. 19; see Fig. 9/II), whereas a much weaker retaining current ( $-5$  nA) totally abolished spontaneous release from the  $0.02$  M solution within 20 min of its application (micropipette no. 12, see Fig. 8/II.C.).

#### c. Release in the presence of an ejecting current

Electrophoretic release was measured using a range of ejecting current intensities ( $+12.5$  to  $+200$  nA). The rate of electrophoretic release was linearly related to the intensity of the ejecting current (see Fig. 10/II; filled circles).

The transport numbers calculated for each micropipette are shown in Table 2/II. The mean transport number of NA obtained from all



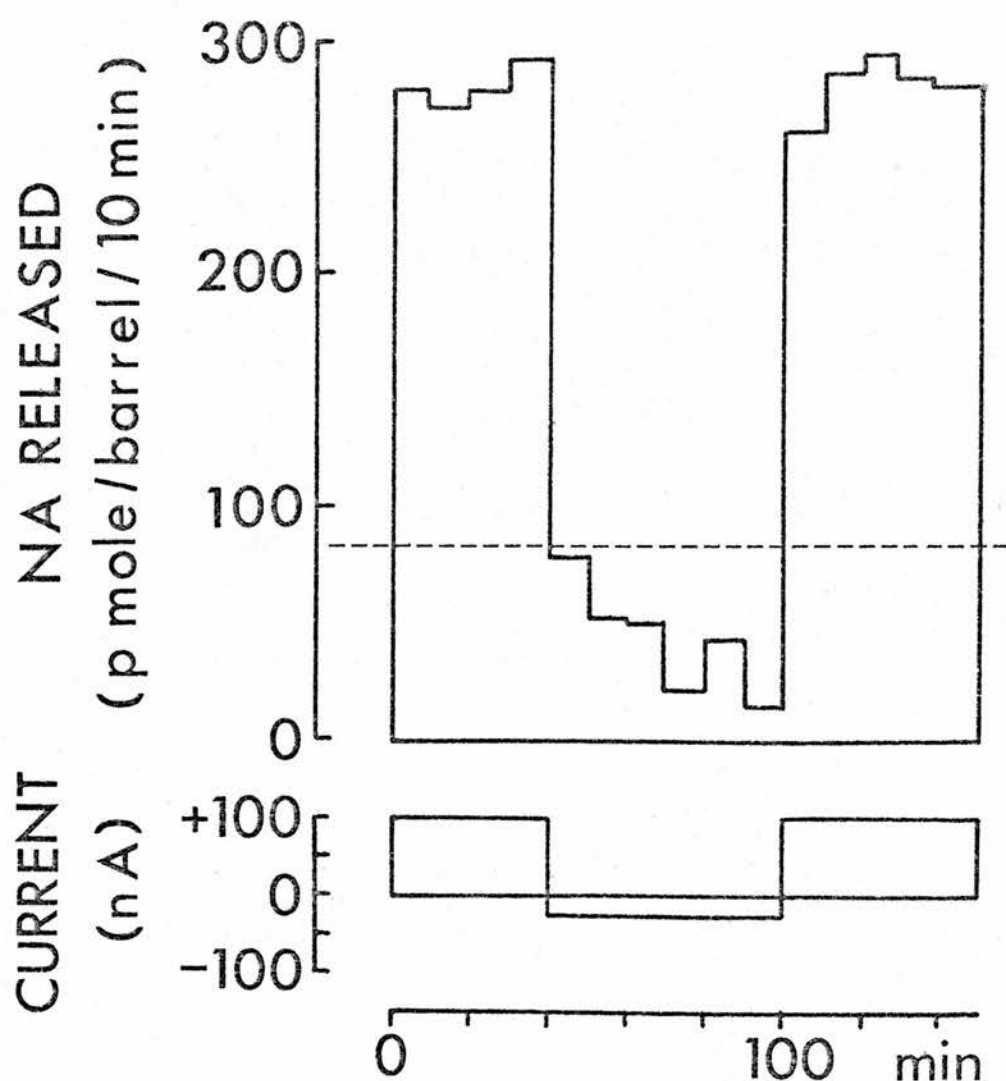


FIGURE 9/II. The effect of a retaining current on the rate of spontaneous release.

Micropipette no. 17 (see Table 2/II). Lower graph: electrophoretic current applied to each of the three barrels containing noradrenaline (NA) (0.2 M, pH 3.5) (ordinate: current intensity, nA, positive upwards; abscissa: running time, min). Upper graph: output of NA during sequential 10 min periods of sample collection (ordinate: output of NA, pmole/barrel/10 min); abscissa: running time, the same scale as in the lower graph). Broken line indicates the steady-state rate of spontaneous release.

The rate of release gradually declined during the application of the retaining current.



five micropipettes was  $0.29 \pm 0.05$ . This value is significantly higher than the mean transport number of NA obtained using the 0.02 M solution ( $0.17 \pm 0.02$ ) (t test;  $p < 0.05$ ).

#### II.3.5. EFFECT OF THE ADDITION OF OTHER IONS

The effect of the addition of other ions upon the release of NA from an 0.2 M solution was investigated by comparing the output from a solution prepared by dissolving NA bitartrate in double glass-distilled water (the pH of this solution was 3.5) with the output from a similar solution to which NaOH had been added in order to raise the pH to 5.0. NaOH was used in these experiments since it had been suggested that in in vivo experiments the electrophoretic application of NA is accompanied by artefactual neuronal responses due to the release of  $H^+$ , and that in order to eliminate these responses it is necessary to raise the pH of the NA solution by the addition of NaOH (Frederickson et al., 1971; see III.B.3).

Some of the characteristics of the five micropipettes used are summarized in Table 2/II. The electrical resistance was lower in the case of barrels containing NA at pH 5.0 (Wilcoxon test:  $p < 0.005$ ).

##### a. Spontaneous release

There was no significant difference between the rate of spontaneous release of NA from the solution at pH 3.5 and from the solution at pH 5.0 (t test,  $p > 0.1$ ).

##### b. Release in the presence of a retaining current

The efficacy of retaining currents was studied in detail using only two micropipettes. The output of NA from the pH 5.0 solution was 2.6 times (micropipette no.17) and 2.5 times (micropipette no. 19) greater

than the output from the pH 3.5 solution during the application of -25 nA for a standard period (40 or 60 min). Thus the retaining current was less effective with the pH 5.0 solution.

#### c. Release in the presence of an ejecting current

Electrophoretic release was measured using a range of ejecting currents (+ 12.5 to + 200 nA). With all the five micropipettes tested, any given ejecting current was less effective in releasing NA from the solution at pH 5.0 than from the solution at pH 3.5. The relationship between current intensity and rate of electrophoretic release for one micropipette is shown in Fig. 10/II. The transport number of NA was calculated individually for each sample collected from each micropipette. The mean values for the transport number obtained from each micropipette are shown in Table 2/II. In every case the transport number of NA was lower when release was measured from the pH 5.0 solution. The overall mean values from the five micropipettes were  $0.29 \pm 0.05$  (pH 3.5 solution) and  $0.11 \pm 0.03$  (pH 5.0 solution); the difference between these two values is statistically significant (t test:  $p < 0.01$ ).

### II.4. DISCUSSION

In the first part of the Discussion the present results are interpreted in terms of the theoretical model of ion movements and in terms of the theoretical 'release curve'. In the second part of the discussion some practical implications of the results are considered.

## RATE OF RELEASE OF NA

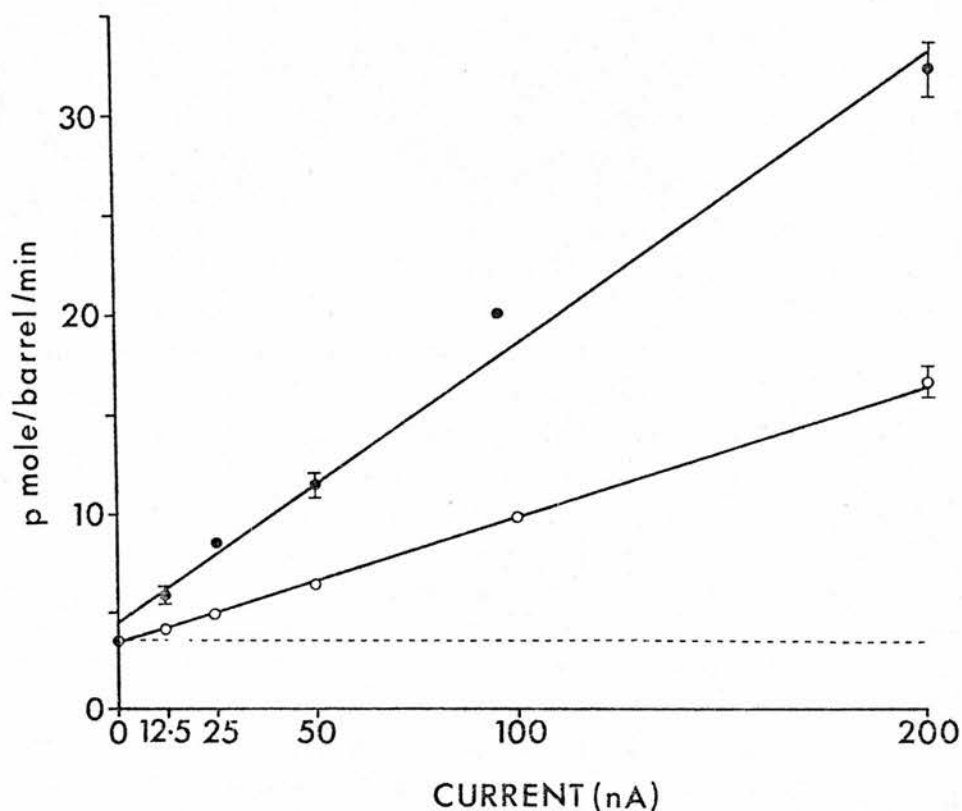


FIGURE 10/II. Rate of release of noradrenaline at different pH values.

Micropipette no. 17 (see Table 2/II). Ordinate: rate of release of noradrenaline (NA) (pmole/barrel/min); abscissa: intensity of ejecting current applied to each of the three NA-containing barrels. Closed circles: release from barrels containing NA at pH 3.5; open circles: release from barrels containing NA at pH 5.0. Each point is the mean of four measurements (standard error is indicated except when it was less than  $\pm 0.25$  pmole/barrel/min. Both lines were obtained by linear regression. The rate of spontaneous release is indicated by the broken line.

The total rate of release of NA is greater from the solution at pH 3.5 than from the solution at pH 5.0.

#### II.4.1. INTERPRETATION OF THE RESULTS ON THE BASIS OF THE MODEL

##### a. Release in the absence of an electrophoretic current

In our experiments, the tip of the micropipette was broken (i.e. the surface area of the tip orifice was increased) immediately before spontaneous release was measured. The increase in the size of the tip orifice would upset the steady-state diffusional relationship which presumably existed before the tip was broken. Thus it would take some time before an interphase layer of unchanging thickness could be re-established at a different level (Fig. 1/II). This could explain the finding that, after the tip of the micropipette had been broken, the rate of release first gradually decreased until a steady-state rate of release was established (Fig. 4/II).

Diffusional leakage from micropipettes can be significant. Using a 0.02 M NA solution, the average rate of spontaneous release was 0.36 pmole/barrel/min, which is approximately 10% of the total rate of release during the passage of +25 nA. However, when a 0.2 M solution was used (as is often the case in in vivo experiments), spontaneous release (8.8 pmole/barrel/min) accounted for as much as 65% of the release in the presence of +25 nA.

##### b. Release in the presence of a retaining current

The model proposes that drug retention is a time-dependent process. The results obtained in our experiments are in agreement with this proposition. Fig. 8/II.C shows that a weak retaining current gradually became completely effective during the period of retention (see also Fig. 9/II). The results shown in Fig. 5/II also support experimental predictions derived from the

model: as higher retaining currents were tested, the amount of drug released during the first 16 min retention was progressively reduced. Since there was no release at all in the presence of -25 nA it is possible to conclude that this current abolished release as soon as it was passed.

According to equation (10)  $t_{\min}$  is directly proportional to the square of the concentration of the drug solution contained in the micropipette. Thus, any given retaining current should be less effective in counteracting release from more concentrated solutions. Fig. 9/II offers qualitative confirmation of this expectation: in contrast to the micropipette shown in Fig. 5/II (0.02 M), Fig. 9/II shows that even after 50 min a retaining current of -25 nA had not succeeded in completely abolishing spontaneous release from a micropipette containing a 0.2 M solution (the two micropipettes had similar tip diameters).

Equation (10) also indicates that retaining currents should take longer to become effective if the transport number of the drug ions is reduced. This prediction is also confirmed by the present experiments (see II.3.5.).

The fact that any given retaining current was less effective if it followed an ejection period than if it followed a period of spontaneous release (Fig. 5/II) lends support to the concept of 'after-diffusion' (Castillo & Katz, 1957), and is readily explicable in terms of the model. During steady-state electrophoretic release the bulk of the solution is assumed to be at the tip boundary (Fig. 3/II). When an ejecting pulse is followed immediately by the passage of a retaining current, the boundary of the bulk of the solution has to migrate through a relatively greater distance ( $\delta_c$  compared to  $\delta_c - \delta_o$ ) before that particular retaining

current becomes effective. Thus it is suggested that the reduced efficacy of a retaining current after an ejecting pulse is a reflection of the prolongation of  $t_{\min}$ .

c. Release in the presence of an ejecting current

The transport number of noradrenaline. As a preceding retaining pulse can interfere with the release during an ejection period (see below), it was necessary to examine the electrophoretic release when no retaining current had been applied previously. In these experiments, there was a linear relationship between the intensity of the ejecting current and the rate of drug release (Figs. 6/II and 10/II). This shows that drug release, under these circumstances, can be described by Faraday's Law. The data obtained from these experiments were used to calculate the transport number of NA. When transport numbers are calculated, it is necessary to know the contribution of spontaneous release to total release. Our practical procedure was to subtract the steady-state rate of spontaneous release from the total rate of release during an ejection period. This was, however, a necessary simplification since the application of the ejecting current may result in local changes in the concentration of drug ions inside and outside the tip, which in turn would alter the rate of diffusional release.

The mean transport number of NA (0.02 M NA bitartrate, pH 3.0), for the 13 micropipettes tested, was 0.17. When a more concentrated solution (0.2 M NA bitartrate, pH 3.5) was used, however, the transport number was considerably greater. A possible reason for this difference is that there were proportionately fewer 'foreign' ionic species in the stronger NA solution.

Other workers have determined the transport number of NA on the basis of electrophoretic release experiments, using a NA hydrochloride solution. Values of 0.34 and 0.37 were obtained from two micropipettes (1.7 M, pH 3.0-4.0) using a fluorimetric method for the assay of NA (Krnjević et al., 1963a), and values of 0.09 (0.03 M, pH 5.5), 0.19 (0.3 M, pH 5.5) (Bradley & Candy, 1970), and 0.05-0.30 (0.5 M) (Hoffer et al., 1971a) were obtained using a liquid scintillation method for the assay of [ $^3\text{H}$ ]-NA. Our results are within the range of values obtained by these workers. More precise comparisons, however, are not possible because of the use of different experimental parameters in these studies (e.g. different NA salt, [ $^3\text{H}$ ]-labelled NA, the application of retaining currents between ejecting pulses).

The effect of retaining currents. It has been suggested (Curtis, 1964) that 'the use of an excessively strong retaining current for prolonged periods .... (may) .... prevent the substance under test from being ejected from the micropipette during the application of short current pulses'. Our results confirm this suggestion (Fig. 7/II).

In order to avoid this unfortunate effect of strong retaining current, Curtis (1964) has recommended the use of a 'just-adequate' retaining current, i.e. a retaining current which exactly counteracts spontaneous leakage without interfering with subsequent electrophoretic release. However, the concept of such a 'just-adequate' retaining current is incompatible with the proposed model, according to which every retaining current, however weak, will eventually become completely effective in counteracting diffusional efflux. Furthermore the model proposes that if a retaining current is passed beyond the point in time at which it successfully counteracts diffusional efflux (minimal effective retention time,  $t_{\min}$ ) it will necessarily begin to deplete the tip of the micropipette of drug ions, thus inevitably interfering with electrophoretic release during the next ejection period.



The model is apparently vindicated by the present results. Fig. 8/II shows that when no retaining current had been applied previously, the release curve was rectangular in shape. The effect of a pre-ejection retaining current was to prolong the rising phase of the release curve. Both the intensity (Fig. 8/II.B) and the duration of passage (Fig. 8/II.A) were important in determining the magnitude of this distortion, thus supporting the proposal that drug retention is a time-dependent process. Fig. 8/II.C. shows that a weak retaining current (-5 nA) was not initially effective in counteracting spontaneous release, but became effective before the end of the fourth 5 min collection period. (Thus,  $t_{\min}$  for this retaining current was between 15 and 20 min.) The continued application of this current for a further 60 min resulted in a significant distortion of the release curve during the subsequent ejection period. (Clarke et al. (1973), measuring the release of radioactive sodium ions, also found a distorting effect of retaining currents on subsequent electrophoretic release.)

#### II.4.2. SOME PRACTICAL IMPLICATIONS

##### a. Contribution of spontaneous release

The estimation of dose in microelectrophoresis experiments is based on Faraday's Law. Faraday's Law, however, applies only to drug ions released from the micropipette by iontophoresis. Even if we are prepared to neglect the contribution of electro-osmosis, there still remains the awkward problem of diffusional release which is necessarily an unknown factor in the in vivo situation. One solution has been proposed by Curtis et al. (1971): they suggest that the rate of spontaneous release may be assessed in terms of the magnitude of the retaining current needed to curb it. Thus, these workers have added the intensity of the 'just-adequate'



retaining current to the intensity of the ejecting current in order to calculate the 'true' dose of drug applied. Since, however, drug retention is a time-dependent process (see above), this procedure seems to be theoretically unjustified. There would seem to be no easy answer to this problem. However, it may be possible to circumvent it by using very dilute drug solutions, so that the contribution of diffusion to total release is relatively very small. The rate of release would then be almost entirely iontophoretic, and thus governed by Faraday's Law.

#### b. Use of retaining currents

The present results indicate that if it is intended to deliver standard pulses of a drug, it is essential that not only the intensity and the duration of the ejecting pulses, but also the intensity and duration of the retaining pulses are kept scrupulously constant. Furthermore, the results shown in Fig. 7/II suggest that after a protracted retention period it may be necessary to apply standard ejecting pulses several times before they can be expected to result in standard pulses of drug release.

#### c. Introduction of 'foreign' ions

The results of these experiments show that the transport number of noradrenaline was lower when noradrenaline was released from a pH 5.0 solution compared to the transport number obtained with a pH 3.5 solution (see II.3.5). The lower transport number of noradrenaline in the pH 5.0 solution is most likely to be due to the introduction of  $\text{Na}^+$  into the solution when the pH was adjusted. By titrating with 1 N NaOH a 0.2 M noradrenaline bitartrate solution, we have found that it behaves as a buffer within the

pH range 2.0-5.0. This reflects the two  $pK_a$  values for tartaric acid (2.98 and 4.34; see Weast, 1972). Thus approximately 110 times more NaOH had to be added to the pH 3.5 solution in order to raise the pH to 5.0 than would have been predicted if only the  $H^+$  existing at pH 3.5 had been titrated. Indeed, the concentration of  $Na^+$  in the final solution at pH 5.0 was 0.1 M.

The reduction in the transport number brought about by the addition of other ions is reflected in a diminished efficacy of both ejecting and retaining currents. The addition of extraneous ions should therefore be avoided wherever possible. In the case of NA there would seem to be no advantage to the practice of raising the pH by the addition of NaOH (see III.B.3). However, the addition of NaCl may be necessary in order to assist the passage of electrophoretic currents when poorly ionised compounds are applied (e.g. see Curtis et al., 1971).

CHAPTER III

THE TIME-COURSE OF NEURONAL RESPONSES

### III.1. INTRODUCTION

It has been known for a long time that the time-course of the neuronal response to a microelectrophoretically applied drug is characteristic of the drug applied (see Curtis & Crawford, 1969). This has been taken as suggestive that the time-course of responses could yield information about the nature of the drug-receptor interaction, and possibly about the physiological role of the drug (e.g. Yamamoto, 1967; Bradley, 1968; Stone, 1972b). Such conclusions cannot be drawn, however, until it is known to what extent physico-chemical processes (release, diffusion) contribute to the time-course of the response.

In an in vitro study, using [ $^{14}\text{C}$ ] -noradrenaline (see Chapter II), we have shown that ejecting and retaining currents interact in determining the shape of the release curve (rate of release plotted against time) during an ejection period. These experiments have demonstrated that a prior retaining current delays the onset of drug release, and prolongs the time necessary to establish a steady-state rate of release. The experiments described below (III.3.) examine how the distortions in the release curve appear in the time-course of the neuronal response.

As a framework for the discussion of the results I shall use some theoretical considerations presented in this Introduction.

#### III.1.1. RELATIONSHIP BETWEEN DOSE AND RESPONSE

The relationship between dose and response in microelectrophoresis experiments can be broken down into the following steps:

- I. ejecting current pulse ('dose')  $\longrightarrow$  drug release;
- II. drug release  $\longrightarrow$  concentration changes at receptors;
- III. concentration changes  $\longrightarrow$  neuronal response.

In the previous chapter I have discussed the relationship between ejecting current pulse and drug release (step I). In this Introduction I shall discuss first the relationship between release and concentration changes (step II), and then the relationship between concentration changes and neuronal responses (step III).

### III.1.2. THE 'CONCENTRATION CURVE'

The 'concentration curve' (concentration plotted against time) describes how the concentration changes in time at a given point in the external medium during the passage of an ejecting current pulse. If, for the sake of simplicity, we ignore the contribution of biological factors (e.g. active transport, diffusional barriers, etc.), we can use a simple physico-chemical model in order to define the relationship between drug release and concentration attained in the tissue. The assumptions underlying this model were put forward by Curtis, Perrin & Watkins (1960). The basic assumptions are: 1. the tip of the micropipette is a point source; 2. the brain tissue is a homogenous medium; and 3. drug ions are released by iontophoresis, but spread in the tissue by diffusion. These assumptions enable us to use the equations derived for the conduction of heat from a point source in solids (Carslaw & Jaeger, 1959).

The equation proposed by Curtis et al. (1960) indicates that there is a close relationship between steady-state rate of drug release from the micropipette and the concentration (c) attained at any distance d from the tip:

$$c = \frac{R_i}{D\sqrt{\pi}d} \cdot \operatorname{erfc} \frac{d}{4\sqrt{Dt}} \quad (1)$$

where  $R_i$  is the rate of iontophoretic release,  $D$  is the diffusion coefficient, and  $t$  is time measured from the onset of the ejecting pulse. If the ejecting pulse is sufficiently long (i.e.  $t \longrightarrow \infty$ ), equation (1) simplifies to

$$c = \frac{R_i}{D\sqrt{\pi}d} \quad (2)$$

(see Curtis, 1964). Equation (2) indicates that during the prolonged passage of an ejecting current, a steady-state concentration should eventually be attained at any given distance from the tip of the micropipette. This steady-state concentration would reflect a balanced relationship between arrival of drug molecules at the fixed point in the external medium and diffusion away from that point. Since Faraday's Law is applicable to describe the release of a drug from a micropipette during the passage of an ejecting current, under steady-state conditions (see II.3.3.), we now have a linear relationship between intensity of ejecting current ( $i$ ), rate of iontophoretic drug release ( $R_i$ ), and the concentration achieved at a fixed point in the external medium ( $c$ ):

$$c = k_1 R_i = k_2 i \quad (3)$$

where  $k_1$  and  $k_2$  are constants.

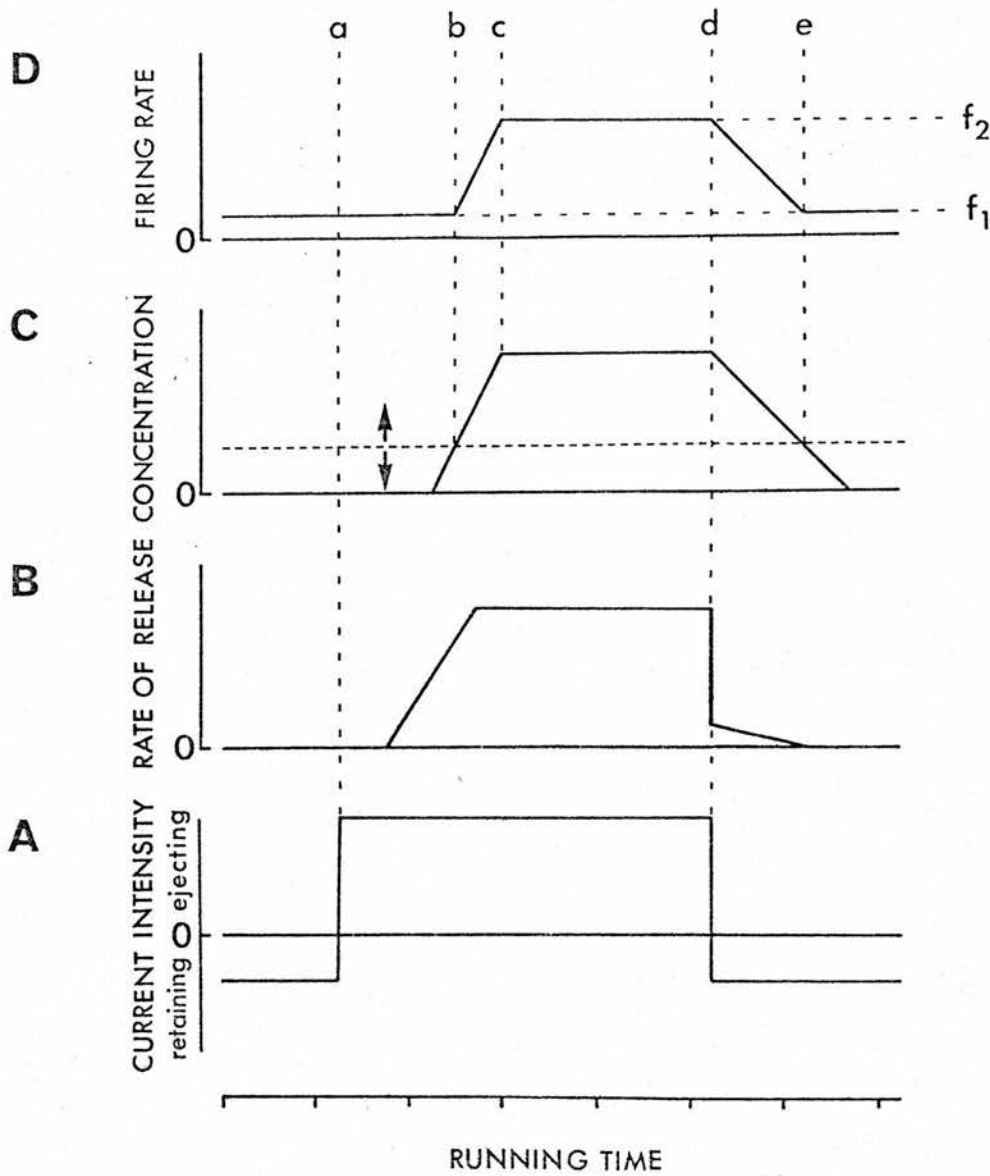


FIGURE 1/III. Hypothetical relationship between ejecting current pulse (A), total release curve (B), concentration curve (C), and response curve for an excitatory response (D).

Horizontal broken line in C indicates level of threshold concentration; arrows indicate the direction of the shift of the threshold in the presence of an antagonist (upward) or a protagonist (downward). Small letters indicate response parameters: a: onset of ejecting pulse; b: onset of response; c: establishment of plateau firing rate; d: termination of ejecting pulse; e: offset of response;  $f_1$ : baseline firing rate;  $f_2$ : plateau firing rate.

Equation (1) enables us to make some predictions about shape of the concentration curve at distance  $d$  from the tip of the micropipette (see Fig. 1/III.C.). During an ejection period, the concentration first increases with time before an equilibrium concentration is attained. The equilibrium concentration, which is directly proportional to the rate of drug release, will be maintained until the steady-state iontophoretic release continues. When iontophoretic release is terminated, the concentration curve will decline at a rate which depends on diffusion.

In Fig. 1/III.C. no assumptions are made about the actual shape of the ascending and descending phases of the concentration curve, and, for the sake of simplicity, both phases are indicated as linear functions of time. There are, however, theoretical reasons for believing that there would be an exponential relationship between time and concentration during the rising and the declining phases of the curve. During these phases, the concentration changes would be described by Fick's second law of non-steady state diffusion, and thus would follow first-order kinetics (see Bockris & Reddy, 1970; Goldstein, Aronow & Kalman, 1974). These theoretical predictions have been confirmed by recent experiments using ion-specific micro-electrodes. The use of these electrodes enables the experimenter to record the entire concentration curve in the external medium during the iontophoretic application of a substance. According to such measurements, the concentration curve has an exponential rising phase, a plateau, and an exponential declining phase. (Morris & Krnjević, 1974; Heineman, Lux & Gutnick, 1977).



It is predicted that changes in the kinetics of drug release would also appear in the concentration curve (see Fig. 1/III):

1. Effect of retaining currents. If the rate of drug release does not rise instantaneously to a plateau level, as is the case after the passage of a prior retaining current (see II.3.3.), it will take a longer time to establish the equilibrium concentration at a point in the external medium. The declining phase of the concentration curve will be influenced by the efficacy of a post-ejection retaining current in counteracting diffusional release after the termination of the ejecting pulse.

2. Effect of ejecting currents. When the same drug is applied with ejecting currents of successively increasing intensities, the concentration curve is likely to be altered in the following way: the latency should become shorter (reflecting the shortening of the release latency), the plateau higher, and the decay time longer (as it would take a longer time for diffusion to reduce the concentration from a higher level to zero).

3. Effect of the mobility of the drug ions. The shape of the concentration curve will reflect the absolute mobility of the drug ions. Let us compare the hypothetical concentration curves for a more mobile drug (A) and a less mobile drug (B), released under identical conditions (the same molarity of the drug solution, the same ejecting current, the same condition of the 'concentration layers' within the micropipette before the start of the ejecting pulse). The release curve of drug A will have a shorter latency, a steeper rise, and a higher plateau, due to the higher transport number of A. All these

features will be reflected in the concentration curve of drug A, which in addition will have a faster declining phase, due to the higher diffusion coefficient of A. Thus the 'fast' time course (short latency, steep rise, high plateau, fast decline) of the concentration curve of drug A contrasts with the 'slow' time course of the concentration curve of drug B (longer latency, less steep rise, lower plateau, long decline).

It must be emphasised at this point that this discussion of the concentration curve has been concerned only with the contribution of physical factors. In vivo, the concentration curve may be modified by biological factors such as diffusional barriers and pathways, active transport mechanisms, enzymic degradation, etc.

### III.1.3. THE 'RESPONSE CURVE'

A neurone can 'respond' to the presence of drug molecules by a change in its electrical excitability. If intracellular recording is used, it is possible to observe the graded membrane response. In the case of extracellular recording (as in the experiments described in this thesis), one can only observe the unitary action potentials, and use the frequency of action potential generation as an index of cellular excitability. Central neurones studied in vivo are often spontaneously active due to their afferent synaptic inputs. Thus the response of a single neurone to a microelectrophoretically applied drug can be either an increase (excitation) or a decrease (depression) in its firing rate. The time-course of this change is described by the response curve (firing rate plotted against time; see Fig. 1/III.D.).

The response curve (for an excitatory response) consists of three main parts: 1. rising phase (including the response latency); 2. plateau; 3. declining phase. (The 'depressant' response curve is a mirror image of the 'excitatory' curve).

It is possible to make some predictions about the relationship between concentration curve and response curve if we make the following simplifying assumptions: 1. there is only one homogeneous receptor population; 2. the receptors are localized at one point in the tissue; 3. the receptors respond instantaneously to a given drug concentration (see Cuthbert & Dunnant, 1970); 4. there is a fixed relationship between concentration change and change in firing rate. It follows from these assumptions that the response curve should closely follow the concentration curve, and any change brought about in the concentration curve (e.g. by manipulating drug release) should also appear in the response curve.

The hypothetical relationship between current pulse (A), release curve (B), concentration curve (C), and the response curve (D) is illustrated in Fig. 1/III. A rectangular ejecting current pulse (A) may result in the release curve shown in line B: the rising phase of the curve may be distorted by a pre-ejection retaining current, and the declining phase may be distorted by diffusional release (see Chapter II). These changes are reflected in the concentration curve (C). The onset of the response (D,b) indicates the presence of a threshold concentration of drug molecules which elicits a detectable change in firing rate. As the concentration rises, the firing rate gradually increases (in the case of an excitatory response) (b - c), and the establishment of a steady concentration will be reflected in

the attainment of the plateau firing rate (c). The declining phase of the concentration curve is reflected in the declining phase of the response curve (d - e), and when the concentration falls to the threshold level, the baseline firing rate recovers (e).

It is possible to derive some experimental predictions from the hypothesis about the close relationship between concentration curve and response curve:

1. Physical factors (release, diffusion) which influence the concentration curve should also influence the time course of the neuronal response.

2. Drugs may modify the response curve in two ways:

- a. by altering the concentration curve (e.g. uptake inhibitors);

- b. by altering the threshold concentration, without changing the concentration curve (see Fig. 1/III.). It is expected that drugs which raise the threshold concentration (antagonists) would increase the response latency, and reduce the recovery time of the response, as well as reducing the height of the plateau. On the other hand, drugs which lower the threshold (protagonists) should shorten the latency, and prolong the recovery time of the response, as well as increasing the height of the plateau.

The experiments described in Section III.3. examine the first group of predictions. Some of the experimental evidence obtained from drug-interaction studies (see later Chapters) will be interpreted by using the second group of predictions.

### III.2. METHODS

#### III.2.1. PREPARATION OF THE ANIMALS

Cats of either sex weighing between 2.0 and 3.5 kg were used in these experiments. Two different techniques were used to prepare the animals.

(a) In earlier experiments the cats were prepared according to the methods of Roberts & Straughan (1967). Anaesthesia was induced using halothane (approximately 2.5%) delivered via a face mask, and was maintained throughout the experiment with halothane (about 1%) delivered via a tracheostomy cannula with an Ayer's T-piece-like arrangement. Catheters were inserted into the cephalic vein and femoral artery for the injection of drugs and recording of blood pressure. ECG was continuously monitored. Two silver ball electrodes were inserted through burr holes in the skull contralateral to the site of single unit recording for monitoring epidural EEG. Rectal temperature was maintained between 37° and 38°C with a heating pad controlled by a thermosensitive rectal probe.

The cat's head was held rigidly in a stereotaxic frame, and the muscles of the back of the neck were cauterised transversely to expose the atlanto-occipital membrane. The membrane was then incised, allowing CSF to run out. A hole about 1 cm in diameter was cut in the skull overlying the anterior sigmoid gyrus, the dura was removed, and 1% agar (at 38°C) was poured into the hole in order to reduce pulsation of the brain during recording.

(b) During the course of the project, several modifications were made to the above techniques.

Under local anaesthesia, a Portex cannula was inserted into the cephalic vein for the injection of thiopentone, which was used to induce general anaesthesia. It was found that this method of induction was less stressful for the animals and obviated the need for high concentrations of halothane during the surgery. High concentrations of halothane were associated with hypotension, cardiac dysrhythmia and raised intra-cranial pressure. After the surgical preparation of the animal had been completed, the administration of thiopentone was discontinued (total amount injected 40-140 mg), and anaesthesia was maintained with halothane, administered via a tracheostomy cannula, for the rest of the experiment. Halothane was delivered from a temperature and flow-rate compensated vapouriser (Fluotek Mk III, Cyprane Ltd.), enabling us to administer a constant concentration of halothane (0.6-1.2%) throughout the experiment. No cell recordings were made until at least 3 hours after the discontinuation of thiopentone.

As well as the continuous monitoring of ECG, EEG, blood pressure and rectal temperature, CO<sub>2</sub> (%) levels at the bifurcation of the trachea were also continuously monitored using a Beckman gas analyser (LB-1). The animals respired spontaneously, inspiring the oxygen-halothane mixture from a reservoir bag via a low resistance non-return valve, and expiring into the atmosphere via another non-return valve. Constant inflation of the inspiratory reservoir bag was maintained by adjusting the flow rate of the oxygen supply. The flow rate thus yielded a

continuous monitor of the respiratory minute volume. Artificial ventilation was used if the spontaneous respiration became irregular, if the respiration minute volume was markedly reduced, or if the end-tidal  $\text{CO}_2$  exceeded 5%. During the course of the experiment a continuous infusion of a dextrose-saline solution (5% dextrose, 0.9% NaCl) was administered at a rate of 4 ml hour, and the bladder was continuously drained using an indwelling catheter.

Modifications were also made to the method of exposing the area of cerebral cortex for recording. A small hole was made in the skull overlying the anterior sigmoid gyrus using a dental burr. An incision was made in the dura (0.5-1 mm in length) under microscopic control, and the arachnoid ruptured using a glass probe, allowing CSF to leak out. The micropipette could then be introduced under microscopic control. This technique has the advantage that it is not necessary to remove CSF and thus the skull behaves as a practically closed box; in this way problems associated with pulsation are minimised.

#### III.2.2. PREPARATION OF THE MICROPIPETTES

The five-barrelled micropipettes used in these experiments were similar to those used in the in vitro experiments (see II.2). One of the five barrels was always filled with 3 M or 4 M NaCl for recording action potentials. In most of the experiments a second barrel was also filled with NaCl for use in 'current balancing' (Roberts & Straughan, 1967). The drug solutions used in the micropipettes were as follows: (-)noradrenaline bitartrate (0.2 M, pH 3.0-3.5); 5-hydroxytryptamine bimalate (0.2 M, pH 3.0-3.5); mescaline hydrochloride (0.2 M, pH 3.5-4.5); acetylcholine chloride (0.2 M, pH 4.0-4.5); carbachol chloride (0.2 M,



pH 4.0-4.5), and sodium glutamate (0.2 M, pH 8.5). With the exception of the sodium glutamate solution, all the solutions were prepared by dissolving the drug salt in double-glass distilled water. The pH of the sodium glutamate solution was adjusted to 8.5 by the addition of NaOH.

### III.2.3. RECORDING OF ACTION POTENTIALS

The recording techniques used in these experiments have been described by Roberts & Straughan (1967). Extracellular action potentials were recorded via a 3 or 4 M NaCl solution contained in one barrel of the micropipette (resistance: 3-10 megohm). The action potentials were amplified using a Bioelectric Electrometer and a Tektronix pre-amplifier, and were fed into an oscilloscope (Tektronix 565) for visual observation. Spikes of equal amplitude were isolated from the noise using a pulse height selector. The pulse height selector generated two sets of standard pulses. One set of pulses was fed back into the oscilloscope to provide a visual monitor of the isolation of spikes from the noise. The other set of pulses was fed into a ratemeter (Ekco, N522C) with a time-constant of 1 sec, and a cumulative recorder (Grass Unit Integrator UI-1). The outputs of the ratemeter were displayed on a pen-writing oscillograph with curvilinear write-out (Grass, model 7).

### III.2.4. MICROELECTROPHORETIC APPLICATION OF DRUGS

Ejecting and retaining currents were provided by the circuit described by Roberts & Straughan (1967). A 300V DC supply was reduced stepwise to obtain voltages, and thus currents, of an appropriate magnitude. The final output resistance of the circuit was 1000 megohm



which minimised any changes in the current flow resulting from eventual changes in the electrical resistance of the drug barrels. The electrophoretic current passed through each drug barrel of the micropipette was continuously monitored using a Pye Scalamp galvanometer.

Artefacts, produced by switching from positive to negative current, were minimised by the use of mercury-wetted reed switches. These were controlled by an independent 12V circuit. The reed switches could be operated either manually or automatically using a sequential timing device (Bevan & Bradshaw, 1973). The current source provided for up to five independent electrophoresis channels.

To control for possible current effects on the cell under study, 'current balancing' was routinely used. This was achieved by passing currents of appropriate polarity and intensity through a barrel of the micropipette containing a 4 M NaCl solution in such a way that a) either the total positive and negative currents passed through all the barrels remained constant during both ejection and retention periods (Roberts & Straughan, 1967), b) or the net current flow through the tip of the micropipette remained zero throughout the experiment (Salmoiraghi & Weight, 1968).

#### III.2.5. RESPONSE MEASURES

In these experiments, action potentials generated by a single unit were defined as spikes of equal amplitude which were clearly separated from the noise level and from spikes of lower amplitude. Pharmacological responses were evaluated on the basis of the ratemeter and cumulative spike counter records. The following parameters were used (see Fig. 2/III):

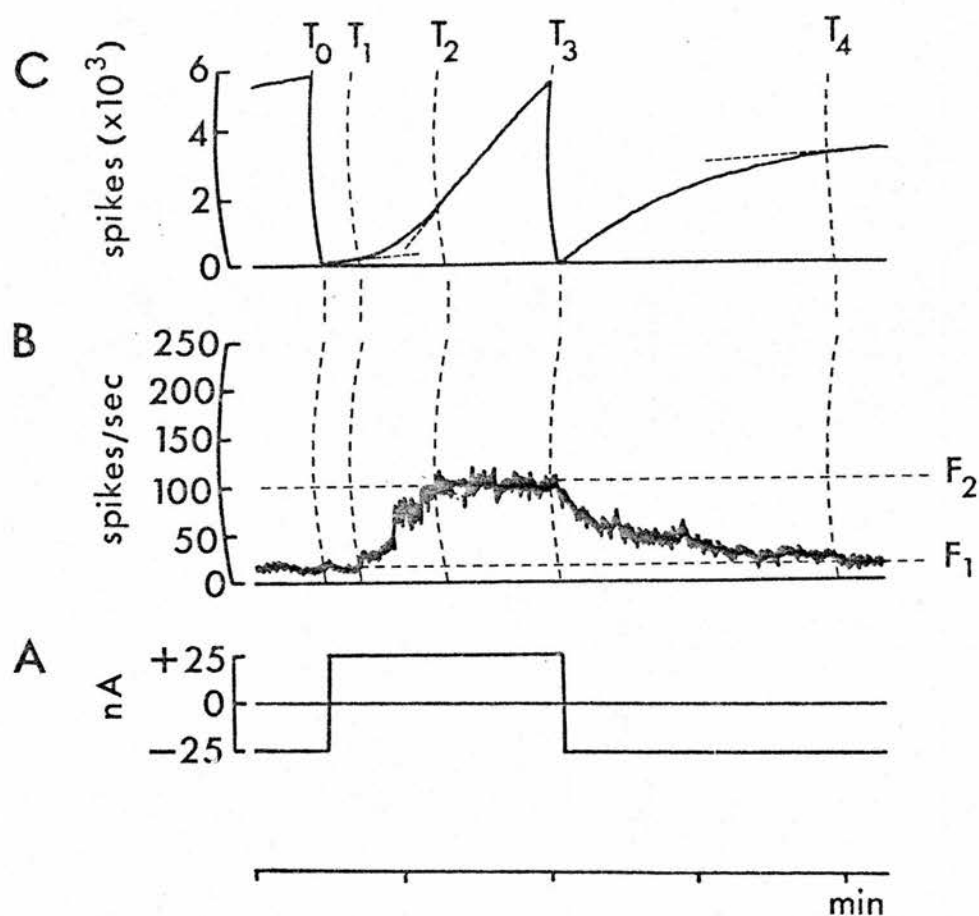


FIGURE 2/III. Parameters of an excitatory response to acetylcholine.

A. Electrophoretic current passed through the solution of acetylcholine chloride (0.2 M) contained in one barrel of the micropipette (ordinate: current intensity, nA, positive upwards; abscissa: running time, min).

B. Ratemeter recording of the firing rate of a single cortical neurone (ordinate: firing rate, spikes/sec; abscissa: running time, min).

C. Cumulative record of the total number of action potentials generated by the neurone (ordinate: total spikes; abscissa: running time, min).

Response parameters are indicated by letters above and to the right of the traces. See text for explanation.

- a) The spontaneous (baseline) firing rate ( $F_1$ ).
- b) The latency of onset of excitation, measured from the onset of the ejecting pulse (response latency) ( $T_0 - T_1$ ).
- c) The maximum (equilibrium) firing rate obtained by the cell during the ejection period ( $F_2$ ). The maximum firing rate was measured under equilibrium conditions, i.e. when prolongation of the ejection period led to no further change in the firing rate. The baseline firing rate was subtracted from the maximum (equilibrium) firing rate in order to obtain the maximum change in firing rate ( $F_2 - F_1$ ).
- d) The time taken to achieve the maximum firing rate ( $T_0 - T_2$ ).
- e) The time taken for the recovery of the spontaneous firing rate after the termination of the ejecting pulse (recovery time) ( $T_3 - T_4$ ).
- f) The total number of action potentials generated in response to the ejecting pulse (total spike number). This measure was used as an index of the magnitude of the excitatory response.

The response latency, the time taken to achieve the maximum firing rate, and the recovery time were estimated by determining the point in time at which the angle of slope of the write-out of the cumulative spike counter stopped changing. This method was found to yield more reliable values than could be obtained by direct measurement of the ratemeter tracing.

The total spike number was either measured directly, using the cumulative spike counter, or was estimated by measurement of the area under the ratemeter tracing, after subtraction of the mean rate of spontaneous firing.

### III.3. RESULTS

All the neurones studied in these experiments were spontaneously active, and in no case was the firing rate controlled by the 'background' application of excitant amino acids. Only excitatory responses were used. In these experiments, we followed the usual practice of passing a retaining current through the drug-containing barrel continuously when ejecting currents were not being passed.

#### III.3.1. THE ROLE OF EJECTING CURRENTS

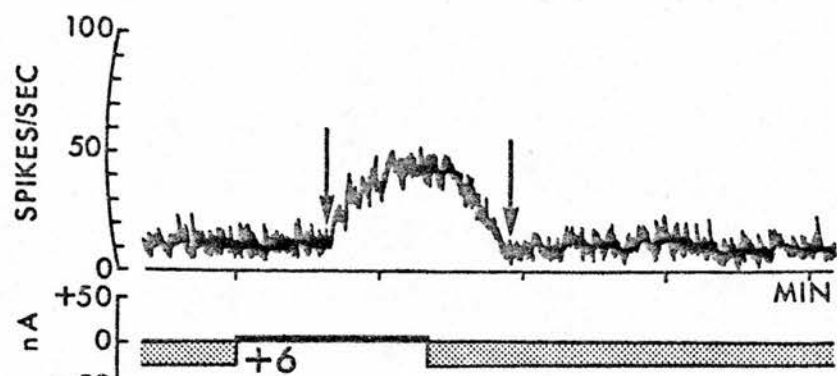
In these experiments, the parameters of the retaining pulse (intensity, and retention time) were kept constant, and the intensity of the ejecting pulse was increased with successive applications.

The following changes in the excitatory responses were observed:

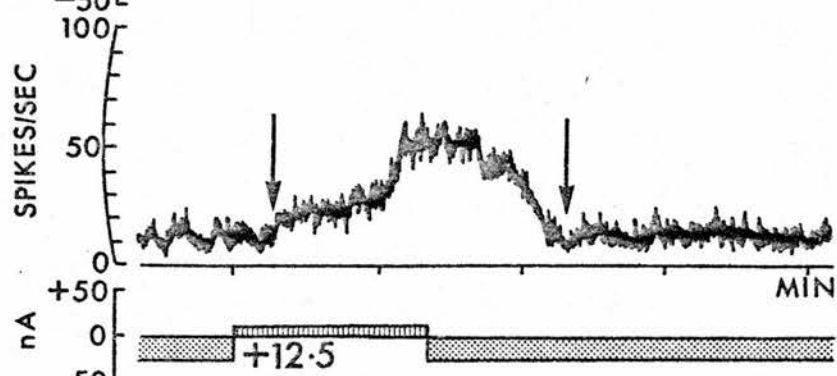
- a. The response latency became progressively shorter.
- b. The latency to plateau became progressively shorter.
- c. The maximum firing rate attained by the cell increased.
- d. The recovery time was prolonged (in the presence of a post-ejection retaining current of standard intensity).
- e. The total spike number increased (when a standard ejection time was used).

Ratemeter recordings of the responses of a single cortical neurone to 5-HT, released from a micropipette with a range of different current intensities, are displayed in Fig. 3/III. Response latencies and latencies to plateau of responses to ACh, carbachol (CCh), 5-HT and NA are shown in Fig. 4/III. Responses to glutamate behaved similarly,

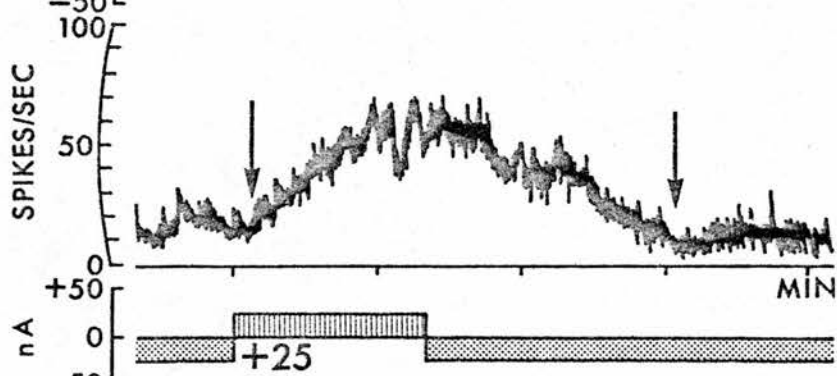
A



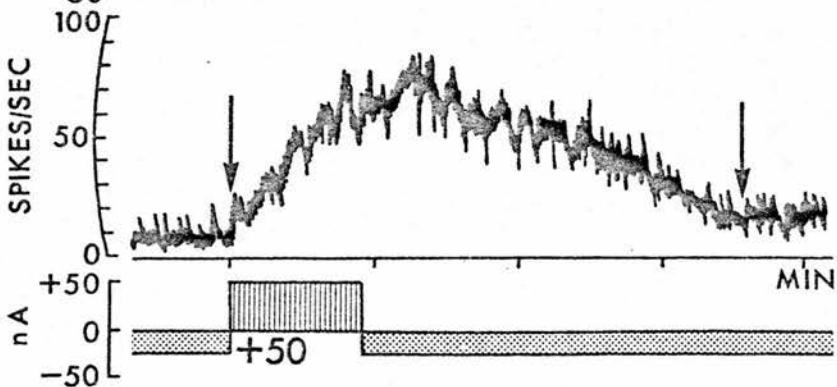
B



C



D



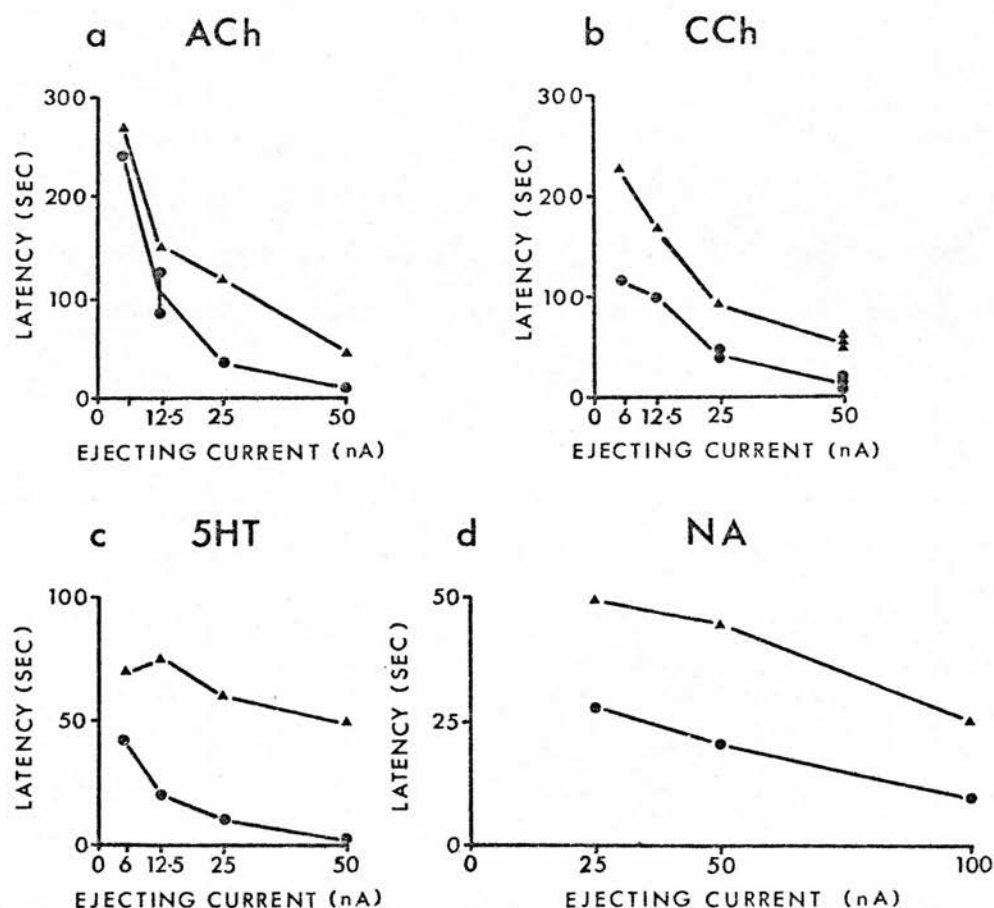


FIGURE 4/III. The relationship between the intensity of the ejection current and the latencies of neuronal responses.

Each graph shows the latencies to onset and latencies to plateau of responses of one individual neurone. Ordinates: latency (sec); abscissae: intensity of ejection current (nA, log scale). Closed circles: latency to onset of the response; closed triangles: latency to plateau.

In the case of all four agonists (ACh: acetylcholine; CCh: carbachol; 5HT: 5-hydroxytryptamine; NA: noradrenaline) the latencies were reduced when higher ejection currents were passed.

but it was not possible to measure the recovery time of these responses, due to their fast time-course.

### III.3.2. THE ROLE OF RETAINING CURRENTS

#### a. Pre-ejection retaining currents

When the intensity of the retaining current applied between ejecting pulses was increased, the response to the standard ejecting pulse was markedly reduced, and, in some cases, completely abolished. This observation is illustrated in Fig. 5/III.

These observations were extended further, using excitatory responses to glutamate. Because of their fast time-course, these responses are particularly suitable for such studies. It was found that a reduction in the size of the response to a standard ejecting pulse could be achieved by increasing the retention time, as well as by increasing the intensity of the retaining current. This observation is illustrated in Fig. 6/III. When the intensity, or duration of application of the retaining current was increased, the reduction in the total spike number was accompanied by an increase in the response latency.

In the studies shown in Fig. 5/III and 6/III, ejecting pulses of standard duration were used. Thus, as the response latency increased following a higher retaining current or a longer retention time, it was not always possible to achieve the plateau firing rate. In order to obtain information about the effect of retaining currents upon other response parameters (plateau firing rate, latency to plateau and recovery time), it was necessary to vary the ejection time according



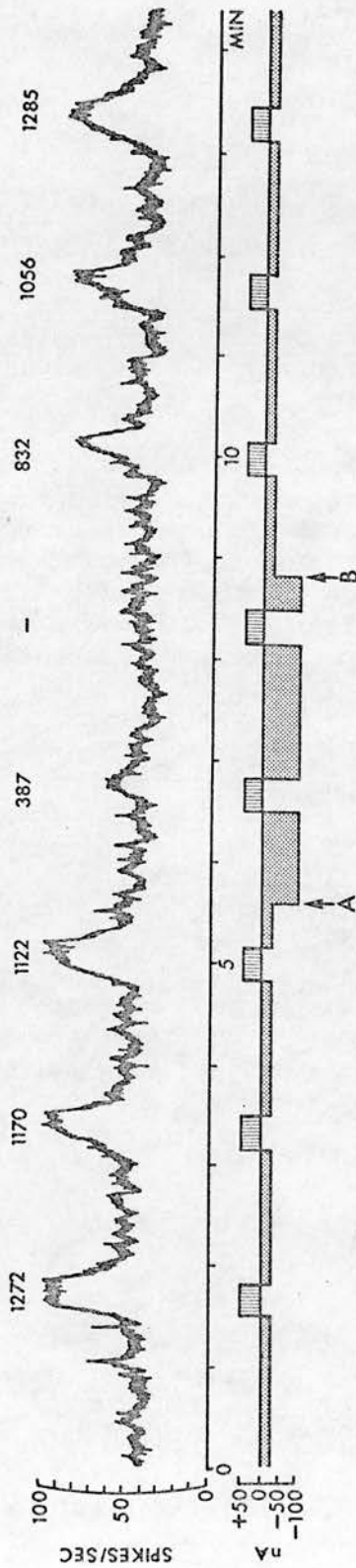
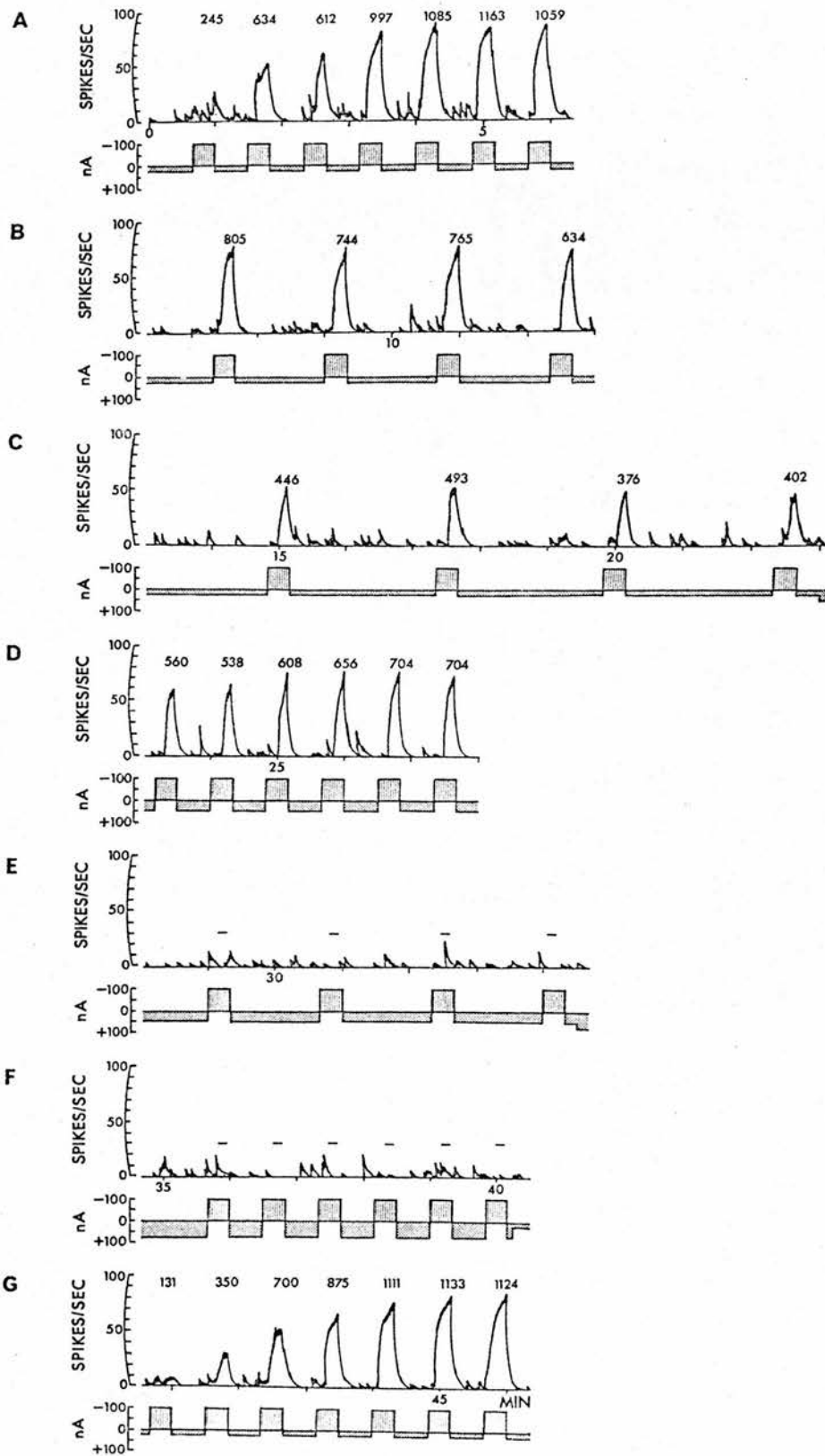


FIGURE 5/III. The effect of an increase in the intensity of the retaining current upon neuronal responses to acetylcholine.

Lower trace: electrophoretic current passed through the solution of acetylcholine chloride (0.2 M) contained in one barrel of the micropipette (ordinate: current intensity, nA, positive upwards; abscissa: running time, min). Stippled area: retaining current; hatched area: ejecting current.

Upper trace: continuous recording of the firing rate of a single cortical neuron (ordinate: firing rate, spikes/sec; abscissa: running time, the same scale as in the lower trace). Figures above the trace represent the total number of spikes generated in response to each ejecting pulse. The parameters (intensity and duration) of the ejecting pulse were kept constant.





to the response. Therefore, following each retention period, an ejecting current of standard intensity was applied continuously for a sufficient length of time to enable the firing rate of the cell to reach an equilibrium value. Such an experiment is illustrated in Fig. 7/III. When either parameter of the retaining current was increased, the response latency and the latency to plateau increased (Figs. 7/III and 8/III), but there was no change in the height of the plateau or in the recovery time in the presence of a retaining current of standard intensity (Fig. 7/III). In the study shown in these figures the retention time was increased: similar observations were made when the intensity of the retaining current was increased.

#### b. Post-ejection retaining currents

After any given ejecting current, the recovery time of the response was constant in the presence of a standard post-ejection retaining current (see previous section). However, when the intensity of the post-ejection retaining current was increased, a reduction in the recovery time was observed, although it was not possible to reduce the recovery time to zero, even when very high retaining currents were applied. An example of this observation is displayed in Fig. 9/III, which shows the recovery times of responses of a cell to ACh.

### III.4. DISCUSSION

The results presented in Section III.3 support the suggestion that the response curve closely follows the concentration curve. The

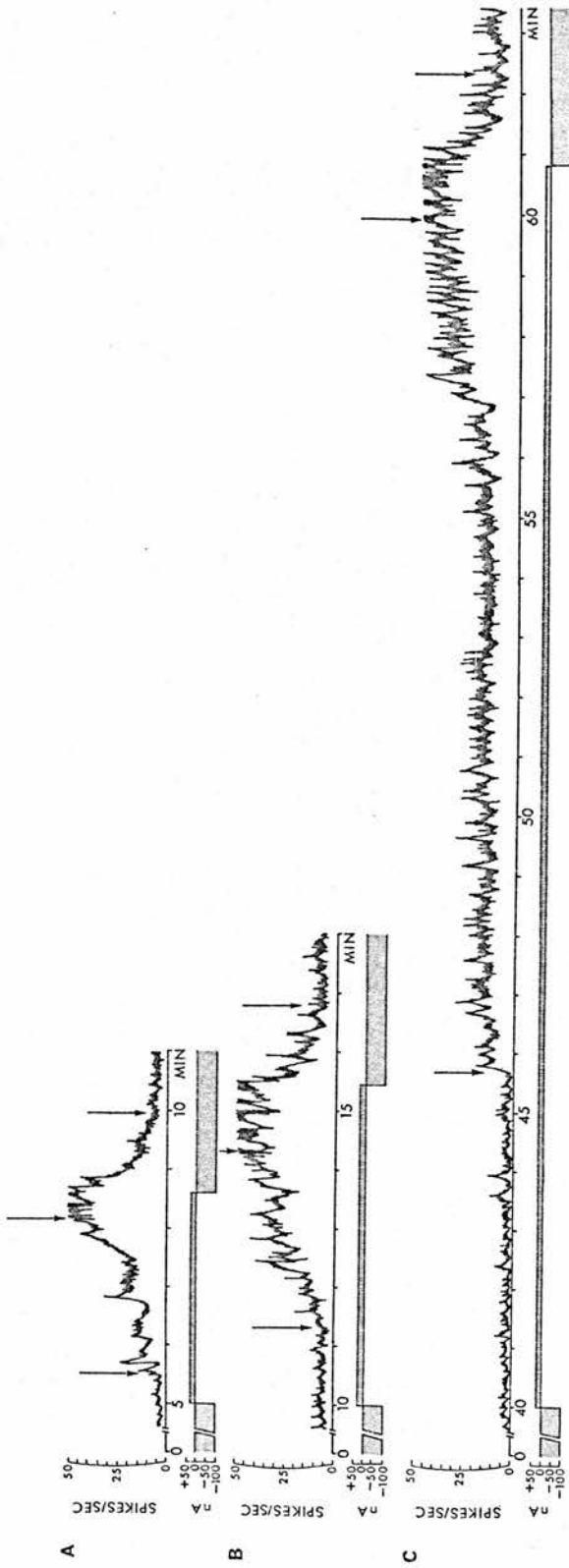


FIGURE 7/III. The effect of changes in the duration of application of the pre-ejection retaining current upon neuronal responses to acetylcholine.

Lower traces: electrophoretic current passed through the solution of acetylcholine chloride (0.2 M) contained in one barrel of the micropipette (ordinates: current intensity, nA, positive upwards; abscissae: running time, min). Stippled area: retaining current; hatched area: ejecting current.

Upper traces: excerpts from the recording of the firing rate of a single cortical neurone (ordinates: firing rate, spikes/sec; abscissae: running time, the same scale as in the lower traces). Arrows above the traces indicate the onset of the response, the attainment of the maximum firing rate, and the recovery of the baseline firing rate. The intensity of the retaining and ejecting currents was kept constant throughout the study, but the retention time was varied.

- A. Pre-ejection retention time: 5 min.
- B. Pre-ejection retention time: 10 min; the response latency and the time taken to attain the maximum firing rate were increased.
- C. Pre-ejection retention time: 40 min; the response latency and the time taken to attain the maximum firing rate were further increased, with no change in the maximum firing rate and the recovery time.

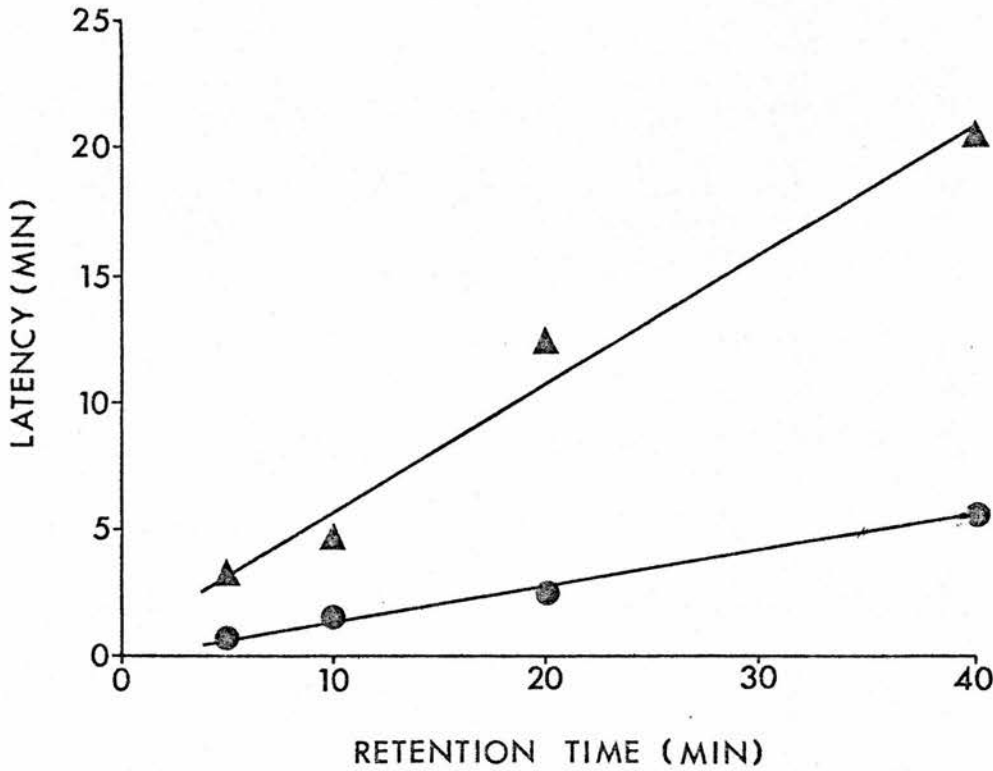


FIGURE 8/III. The effect of retention time on response latency and latency to plateau of the response of a single cortical neurone to acetylcholine.

Closed circles: response latency; closed triangles: latency to plateau. The intensity of the retaining current was  $-100$  nA, and the intensity of the ejecting current was  $+25$  nA.

An increase in the retention time resulted in an increase in the response latency and the latency to plateau.

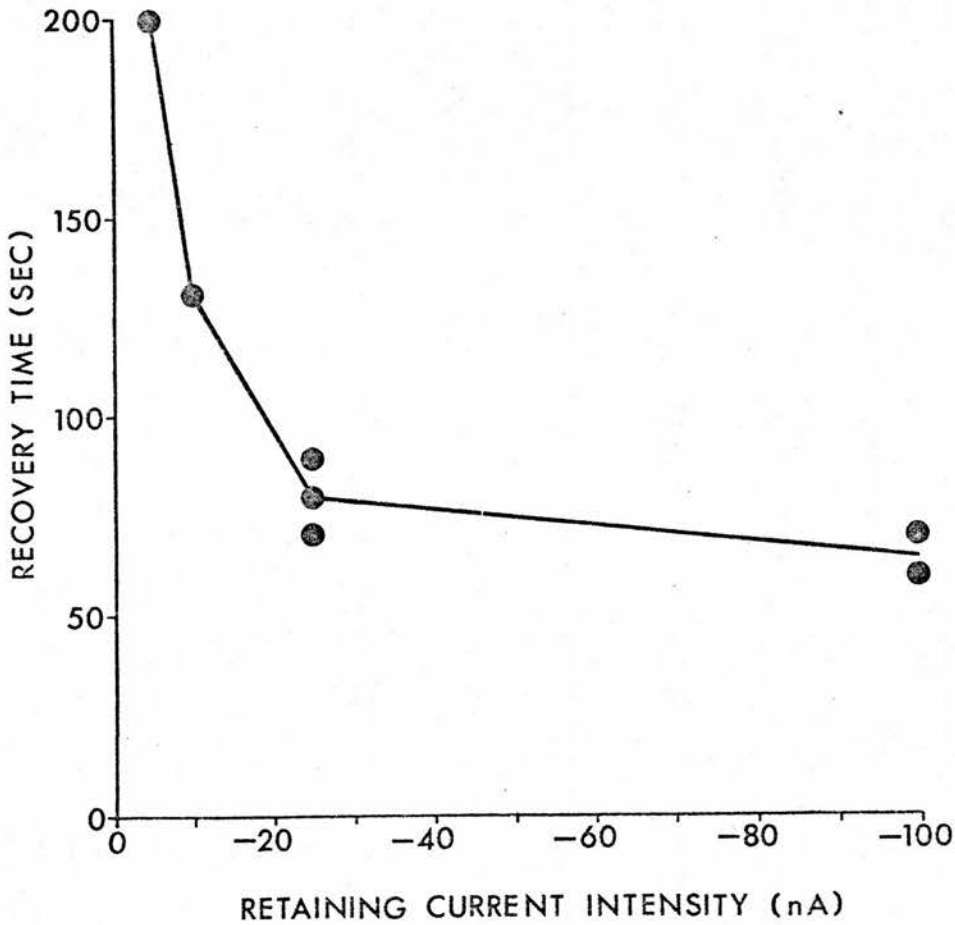


FIGURE 9/III. The effect of the intensity of the post-ejection retaining current upon the recovery time of responses of a single cortical neurone to acetylcholine.

Recovery times were measured in the presence of retaining currents of various intensities following plateau responses to acetylcholine (ejecting current, +100 nA).

The recovery time was reduced when higher post-ejection retaining currents were used.

changes in the response curve observed when increasingly higher ejecting currents were used are consistent with the predictions presented in III.1.2. for the concentration curve: the latency is reduced, the plateau is raised, and the recovery time is prolonged.

Since it is proposed that the concentration curve reflects in part the shape of the release curve, it is to be expected that all the distortions imposed on the release curve by the passage of retaining currents would be manifested in the response curve. This prediction is confirmed by the present experiments:

#### a. Pre-ejection retaining currents

An increase in either the intensity or the duration of the pre-ejection retaining current results in an increase in the response latency and latency to plateau, but does not alter the plateau firing rate itself. These findings are complementary to the observations of the release curve measured in vitro (e.g. compare Figs. 8 /II and 7/III). When standard ejecting pulses are used, the distortions in the response curve are reflected in a decrease in the total spike number; this presumably results from a decrease in the total drug output during the ejection period (compare Figs. 7/II and 6/III).

It is apparent from Fig. 6/III that an increase in the interval between consecutive ejecting pulses can result in the complete abolition of neuronal responses to glutamate. It is obvious, therefore, that if quantitatively reliable responses are to be achieved in a microelectrophoresis experiment, it is essential that each drug being tested is applied at regular intervals throughout the study.

#### b. Post-ejection retaining currents

The post-ejection retaining current can modify the recovery time of the response (Fig. 9/III). The finding that even in the presence of -5 nA the original baseline firing rate completely recovered is supported by the finding that very weak retaining currents which are not capable of abolishing diffusional release instantaneously eventually became completely effective (cf. Fig. 8/II.C.). The progressive shortening of the recovery time in the presence of retaining currents of higher intensities is presumably a reflection of progressive shortening of the minimal effective retention time ( $t_{\min}$ ). The fact that -25 nA was practically as effective as -100 nA suggests that -25 nA almost instantaneously abolished spontaneous release (i.e.  $t_{\min} = 0$ ), and that the recovery time observed in the presence of these currents reflects the reduction in concentration of the drug at receptor sites when there was no diffusional leakage from the micropipette.

The in vivo observations reported in Section III.3. confirm the practical relevance of the in vitro studies for the use of the microelectrophoretic technique under 'normal' experimental conditions. It is important to note that despite the fact that more dilute drug solutions were used in most of the in vitro experiments, the effects of retaining currents on the release curve are paralleled in every way by their effects on the response curve. Retaining currents are universally used in microelectrophoresis experiments. There is little, if anything, to be gained from the use of weak retaining currents,

for if a retaining current is to be effective in counteracting spontaneous release it must necessarily distort the rising phase of the subsequent release curve (and hence also the response curve). The use of a relatively high retaining current has the advantage that it may abolish release promptly and thus allow time for the neurone to recover before the next ejecting pulse. Sustained diffusional release may give rise to subthreshold concentrations of drugs whose presence becomes apparent only in the presence of a protagonist (Curtis et al., 1970); it may also be source of desensitisation (Roberts & Straughan, 1967).

It was mentioned in III.1. that many workers have attributed some biological significance to the different time-courses observed with different drugs. The alternative position considered here is that differences between time-courses of responses to different drugs are to a great extent due to differences between the mobilities of the drug molecules. It was suggested earlier, on theoretical grounds, that the concentration curve of a more mobile drug will have a 'faster' time-course than that of a less mobile drug released under identical conditions (III.1.2.). These hypothetical differences between the concentration curves correlate well with actual differences between the response curves; more mobile drugs tend to evoke 'faster' responses than less mobile drugs (see Table 1/III). It is also relevant that the same drug applied microelectrophoretically in different tissues tends to evoke responses of similar time-courses (Stone, 1971, 1972a). Of course, on the basis of the presently available evidence, one cannot conclude with certainty that physical factors alone are responsible for



TABLE 1/III. Correlation of response kinetics with published values of transport numbers

TIME COURSE <sup>+</sup>	DRUG	RESPONSE	TRANSPORT NUMBER	REFERENCE
'FAST'	GLYCINE	-	0.5	Werman et al. (1966)
	GLUTAMATE	+	0.4	estimated from Zieglängsberger et al. (1969)
'INTERMEDIATE'	ACh	+	0.24, 0.48	Bradley & Candy (1970)
'SLOW'	NA	+,-	0.05-0.30	Hoffer et al. (1971)
			0.09, 0.19	Bradley & Candy (1970)
			0.17, 0.29	present results (see II.3.3.)
	5-HT	+,-	0.4	Krnjević et al. (1963a)
			0.18, 0.31	Bradley & Candy (1970)

<sup>+</sup>The characteristic time-courses of responses evoked by these drugs are described by Curtis & Watkins (1960), Krnjević & Phillis (1963a,b), Hösl & Tebēcis (1970), Kawamura & Provini (1970), Satinsky (1967), Roberts & Straughan (1967), Johnson et al., (1969a), Couch (1970), Tebēcis (1970), Boakes et al. (1970), York (1970), Boakes et al. (1971), Hösl et al. (1971), McLennan and York (1966), Curtis et al. (1971); see also present results (e.g. Figs. 3/III, 5/III, 6/III).

There is a significant positive correlation between the 'speed' of response and the transport number of the drug ion (Spearman Rank Correlation Test:  $r_s = 0.73$ ,  $p < 0.01$ ).

the shape of the response curves. However, any conclusion about the involvement of biological factors (e.g. kinetics of drug-receptor interaction) should be based on an understanding of the contribution of physical factors to the determination of the time-course of neuronal responses.

CHAPTER IV

RESPONSES OF CORTICAL NEURONES TO NORADRENALINE

#### IV.1. INTRODUCTION

The term 'cerebral cortex' refers to the neocortex; archicortical structures (e.g. hippocampus) are described under a separate heading (for review see I.4.). The term 'cortical neurone' usually designates an anatomically unidentified neurone recorded in the frontal (somato-motor) or parietal (somato-sensory) region of the neocortex. When the pharmacology of neurones in the temporal (auditory) or occipital (visual) cortex is studied, these neurones are usually specified by the cortical region studied.

Most of the experimental information available in the literature derives from two species: the cat and the rat. All the earlier experiments were performed with cats, the traditional experimental animal of neurophysiology. More recently, however, mainly due to financial pressures, many laboratories have started using rats for microelectrophoresis experiments. This applies also to the work presented in this thesis: the earlier work has been done with cats, more recent data, however, have been obtained with rats. It will always be specified which species was used for a particular series of experiments.

##### IV.1.1. DIRECTION OF RESPONSES

Similarly to neurones in most other structures of the brain (see I.3.), cerebral cortical neurones can respond with both excitation and depression to NA applied by microelectrophoresis.

a. Cat

In an early study, Krnjević & Phillis (1963c) described a predominantly depressant action of NA in barbiturate anaesthetized cats. Although excitatory responses were occasionally observed when higher ejecting currents were used, these authors suggested that these were probably of a non-specific nature.

Johnson et al. (1969a) described both excitatory and depressant responses in the cerebral cortex of the halothane-anaesthetized cat. These authors found excitations more frequently than depressions, and suggested that probably the excitations represented the genuine pharmacological effects of NA. This suggestion was based on two different experimental observations: 1. the proportion of cells excited was higher in halothane-anaesthetized or unanaesthetized encéphale isolé preparations than in barbiturate-anaesthetized preparations in which depressions predominated (Johnson et al., 1969b); 2. excitatory responses to noradrenaline could be reversibly antagonized by  $\alpha$ - and  $\beta$ -adrenoceptor blocking agents, whereas the depressant responses were relatively insensitive to the adrenoceptor blocking agents (see below).

In 7 cats, anaesthetized with a mixture of methoxyflurane and nitrous oxide, Lake, Jordan & Phillis (1972) described only depressant responses to microelectrophoretically applied NA. In another paper, the same authors (Jordan, Lake & Phillis, 1972c) reported only depressant responses in 6 cats anaesthetized in the same way.

In a series of experiments, using unanaesthetized cats (cerveau isolé or conscious animals, Frederickson et al. (1971; 1972) described both excitatory and depressant responses; however, when the pH of the NA solution was 4.0 or higher (see IV.1.3.), the proportion of cells excited was relatively small (about 10% of all responses).

#### b. Rat

Apart from our laboratory (see Results: IV.3.1.), two other laboratories have investigated the effects of NA on single neurones in the cerebral cortex of the rat: Phillis and his colleagues in Winnipeg and later in Saskatoon, and Stone in Aberdeen. Unfortunately, the results from the three laboratories are not directly comparable due to the use of different anaesthetics: Phillis and his colleagues used a mixture of methoxyflurane and nitrous oxide, Stone used urethane, and we used halothane.

The reports from Phillis' laboratory describe only a depressant action of NA on neurones in the cerebral cortex of the rat anaesthetized with a methoxyflurane-nitrous oxide mixture (Jordan, Lake & Phillis, 1972c; Phillis, Lake & Yarbrough, 1973; Yarbrough, Lake & Phillis, 1974; Phillis, 1974). Stone in his earlier papers described both excitatory and depressant responses to NA on cortical neurones in the urethane-anaesthetized rat (Stone, 1973a; Stone, 1973b; Anderson & Stone, 1974); most of the responses were depressions, and only a small proportion (5 - 10%) of the responses were excitations. In some more recent papers (Stone, 1974; Stone, Taylor & Bloom, 1975) only depressant responses to NA were reported by the same author.

c. Guinea pig

Jordan et al. (1972c) reported only depressant responses to NA in the cerebral cortices of 11 guinea pigs anaesthetized with methoxyflurane-nitrous oxide.

d. Rabbit

Giardina et al. (1973) described both excitatory and depressant neuronal responses to NA in the cerebral cortex of the unanaesthetized rabbit: 50% of the responses were excitations, and 50% were depressions.

e. Monkey

Nelson et al. (1973) described both depressant and excitatory responses to NA in the cerebral cortex of the squirrel monkey anaesthetized with chloralose or halothane. There was a predominance of depressions: only about 5% of the responses were excitatory.

Foote et al. (1975) examined the effect of microelectrophoretically applied NA on acoustically evoked neuronal responses in the auditory cortex of the unanaesthetized squirrel monkey: NA had a depressant effect on these evoked responses.

IV.1.2. THE ROLE OF ANAESTHESIA

In microelectrophoresis experiments, as in most neurophysiological experiments, anaesthetized animals are used. The presence of a chemical anaesthetic is an experimental variable which may well influence the physiological and pharmacological observations made on single neurones. There are three possible ways of avoiding the interpretative dangers arising from the use of anaesthetics:

1. to use unanaesthetized animals paralysed with a muscle relaxant;
2. to use unanaesthetized, unrestrained, awake animals;
3. to use an anaesthetic which interferes least with the physiological and pharmacological observations,

The first alternative has to be rejected on ethical grounds. A compromise seems to be to use 'encéphale isolé' preparations in combination with the muscle relaxant (Johnson et al., 1969b); but in this case the preparation might well be affected by the major surgery, and also by the muscle relaxant (e.g. gallamine). Although microelectrophoretic drug application and recording of single neuronal activity have been tried in unanaesthetized, unrestrained, conscious animals (Frederickson, Jordan & Phillis, 1972; 1973), these preparations are too unstable for the lengthy studies required when the interaction between drugs is investigated. Therefore, at present, the last alternative is the most practicable: i.e. it is necessary to find the 'ideal' anaesthetic which has the minimum detrimental effect on the pharmacological observation. The search for the 'ideal' anaesthetic is based on exclusion: anaesthetics with marked effects on neuronal responsiveness have to be avoided.

There is now ample evidence showing that the barbiturates can alter markedly the pharmacological responses of neurones in the CNS. Bloom et al. (1965) reported that ACh had an excitatory effect on caudate neurones in the unanaesthetized cat which was abolished or converted into a depressant effect in barbiturate anaesthesia. Johnson et al. (1969b) observed that the proportions of cortical neurones excited by NA and 5-HT were



reduced in thiopentone anaesthesia, and that excitatory responses to the monoamines could be abolished by the systemic injection of thiopentone. Microelectrophoretically applied barbiturates were found to abolish responses to homocysteate and to ACh in the cerebral cortex (Crawford, 1970). Barbiturates administered intravenously or microelectrophoretically were found to block selectively excitatory responses of brain stem neurones to ACh (Bradley & Dray, 1973). In an olfactory slice preparation barbiturates were described to depress the sensitivity of neurones to glutamate (Richards & Smaje, 1976); excitatory responses to ACh, however, were less affected (Smaje, 1976).

There are some recent reports suggesting a possible direct postsynaptic inhibitory action for barbiturates. Nicoll et al. (1975) found that barbiturates hyperpolarize hippocampal neurones, and markedly prolong synaptically evoked post-synaptic inhibitory potentials. Barker (1975a; 1975b) reported a similar post-synaptic depressant effect of barbiturates on the crustacean neuromuscular junction and on invertebrate neurones.

There is less detailed information concerning the effects of other anaesthetics on the chemical sensitivity of central neurones. Johnson et al. (1969b) found that halothane, in contrast to barbiturates and chloralose, did not alter the sensitivity and the pattern of responsiveness of cortical neurones to NA and 5-HT. Crawford (1970) reported that chloralose markedly reduced the sensitivity of cortical neurones to ACh and excitant amino acids;

whereas urethane, nitrous oxide, trichloroethylene, methoxyflurane, and halothane had little effect. Bradley & Dray (1970) found that tribromoethanol and urethane did not change the proportions of cells excited or depressed by ACh, 5-HT and NA in the brain stem. In an in vitro preparation of the guinea pig olfactory cortex, Richard & Smaje (1976) observed that ether, methoxyflurane, trichloroethylene, and alphaxolone, similarly to the barbiturates, depressed the sensitivity of neurones to glutamate, whereas halothane did not affect neuronal sensitivity.

Halothane would seem to be a reasonable choice of anaesthetic for the following reasons: 1. halothane has little effect on the chemical sensitivity of central neurones (Crawford, 1970; Richard & Smaje, 1976); 2. halothane does not prevent the occurrence of excitatory responses to NA and 5-HT, which are frequently observed in unanaesthetized 'encéphale isolé' preparations (Johnson et al., 1969b); 3. it is easy to administer halothane in a reliable way to maintain a steady level of anaesthesia for periods of many hours; 4. halothane can be administered in pure oxygen, and thus the danger of hypoxia can be avoided.

Therefore, halothane was used routinely in all our in vivo experiments. We are aware, however, of the possibility that even this 'near-ideal' anaesthetic might have interfered with the neuronal pharmacology observed by us. A further series of experiments is in progress which will repeat some of the pharmacological studies described in this thesis using in vitro recording from brain slice preparations in the absence of any anaesthetic.

#### IV.1.3. THE ROLE OF pH

Krnjević & Phillis (1963a) reported that hydrogen ions, when released from acidic solutions with high ejecting currents for prolonged periods, could, rather unpredictably, excite some cortical neurones. After a systematic study with NaCl solutions at different pH values, these authors concluded that this effect was very unlikely to occur unless the pH of the drug solution contained within the micropipette was lower than 2.5. Nevertheless, this raises the possibility that  $H^+$  released from an acidic drug solution may modify the neuronal response to the drug.

Johnson et al. (1969a) conducted careful controls for the possible effects of  $H^+$  released during the electrophoretic ejection of NA. Since NA released from solutions at pH 3.0 had identical effects to NA released from solutions at pH 5.0, these authors concluded that  $H^+$  was unlikely to play a role in the responses to NA. Their findings have been confirmed by Stone (1972c).

Frederickson, Jordan & Phillis (1971) obtained results which were in direct conflict with those of Johnson et al. (1969a). They reported that NA released from solutions at pH 4.0 or pH 5.0 was depressant. However, if solutions at pH 3.0 or less were used, excitatory responses were frequently encountered. Similar results were obtained with 5-HT (Jordan, Frederickson, Phillis & Lake, 1972a). In subsequent papers, the same authors (Jordan, Lake & Phillis, 1972b; Hewes & Frederickson, 1974) reported that  $H^+$  released electrophoretically from HCl solutions could also excite cortical neurones. These authors concluded that their results 'clearly show that hydrogen ions applied iontophoretically from drug solutions

or from solutions of HCl can cause excitation of neurones, and that such hydrogen ion effects are responsible for a major portion of the excitation observed in the cerebral cortex when NA is applied from acidic solutions' (Jordan et al., 1972b). They further argued that 'the true action of 5-HT (and NA) on cortical neurones ..... is inhibitory'.

In view of the controversy about the nature of excitatory responses to NA, it was decided during the present project to conduct a further investigation of the involvement of  $H^+$  in these responses. The results of these experiments are described below (IV.3.3.).

#### IV.1.4. THE ROLE OF INDIRECT EFFECTS

When a drug is applied by microelectrophoresis, it may have a direct effect on the cell whose activity is being recorded; the drug, however, may affect the electrical activity of the nerve cell in an indirect way by exerting an action on neighbouring structures (e.g. other neurones that are in synaptic contact with the neurone studied, axon terminals of more distant neurones, glia cells, blood vessels). It is often difficult to differentiate between direct and indirect effects, and the evidence supporting an argument either way is often circumstantial.

Stone (1971) suggested that the excitatory responses to microelectrophoretically applied NA might be due to an indirect effect of the drug on neighbouring small blood vessels: NA would cause constriction in the vessel, which in turn would result in hypoxia;

and hypoxia would cause depolarisation of the neurone. This suggestion was based on Stone's observation (Stone, 1971; 1972a) that mesenteric arterioles of the rat are constricted by microelectrophoretically applied NA, and this vasoconstriction has a 'slow' time-course reminiscent of the time-course of the 'slow' excitatory responses to NA.

Stone's argument has been criticised on several grounds. Krnjević (1973) pointed out that cerebral blood vessels were insensitive to NA. Boakes *et al.* (1972) drew attention to the fact that not only excitatory, but also depressant responses to NA often had a 'slow' time-course. Brawley & Johnson (1973) emphasized that it was a crude simplification to talk about 'the latency' of the response to NA; response latency varied considerably from cell to cell, and was related to the baseline firing rate of the neurone. Szabadi & Bradshaw (1972; 1973) pointed out that not only NA evoked 'slow' excitatory responses; similar responses were evoked by 5-HT, isoprenaline, and mescaline, and these drugs did not have an unequivocal vasoconstrictor effect on cerebral blood vessels. These authors also argued that the 'slow' time-course of these responses might reflect the physical properties of the drug molecules rather than some biological feature of drug action (see also III.4. and Table 1/III).

We can conclude, therefore, that, although it is not possible to rule out completely indirect vascular effects in in vivo microelectrophoresis experiments, there are no good reasons for believing that the excitatory responses to NA, in

particular, are due to such effects. A possible vascular contribution to a neuronal response could be eliminated, however, by repeating the experiments in vitro using a tissue slice preparation.

#### IV.1.5. MODIFICATION OF RESPONSES TO NORADRENALINE BY DRUGS

##### a. Adrenoceptor blocking agents

The most comprehensive study involving these drugs was conducted by Johnson et al. (1969a) in the cerebral cortex of the cat. These authors found that the  $\alpha$ -adrenoceptor blocking agents dibenamine, phentolamine, and thymoxamine selectively antagonised excitatory responses to NA (responses to ACh, glutamate or 5-HT were not affected). The effects of phentolamine and thymoxamine were reversible. Depressant responses were usually unaffected by these antagonists; only on one occasion did thymoxamine antagonise the response. The  $\beta$ -adrenoceptor blocking agents propranolol, isopropylaminoitrophenylethanol (INPEA), and sotalol were also effective in antagonising excitatory responses to NA. Propranolol occasionally (on 2 out of 8 cells) antagonised the depressant response to NA; INPEA and sotalol did not seem to be effective in antagonising depressant responses. The authors concluded that the excitatory responses might be mediated by adrenoceptors which are different from peripheral adrenoceptors, and that the depressant responses might be of a 'non-specific' nature.

In agreement with Johnson et al., (1969a), Bevan et al., (1974a) found that the  $\beta$ -adrenoceptor blocking agent, sotalol, was an effective antagonist of excitatory responses to NA in the cerebral cortex of the cat. Sotalol, however, was found to be less specific than was suggested earlier by Johnson et al., (1969a): when the response to NA was antagonised, the response to 5-HT was also abolished; responses to ACh were not affected. Sotalol was also an effective antagonist of excitatory responses to mescaline. When sotalol was applied with a low ejecting current continuously, it often first potentiated the responses to the monoamines, and antagonism occurred only when the 'dose' of sotalol was increased (Bevan et al., 1974a; Bevan et al., 1974b).

Frederickson et al. (1972) reported that, in the cerebral cortex of the cat, the  $\alpha$ -adrenergic blocking agents dibenamine, phentolamine, and phenoxybenzamine were effective in antagonising both excitatory and depressant responses to NA, without affecting depressant responses to GABA; however, responses to 5-HT and ACh were also affected by these antagonists. The  $\beta$ -receptor blocking agent INPEA was also effective in antagonising both kinds of responses to NA.

Stone (1973a) reported that, in the cerebral cortex of the rat, the  $\alpha$ -adrenoceptor blocking agent, phentolamine, was usually unable to antagonise depressant responses to NA; on a few cells, however, phentolamine proved to be effective. The effect of phentolamine was not studied on excitatory responses.



The  $\beta$ -adrenoceptor blocking agents, propranolol and sotalol, were effective in antagonising both depressant and excitatory responses to NA. The author concluded that the depressant responses to NA might be mediated via  $\beta$ -adrenoceptors.

Freedman et al., (1975) observed that the  $\beta$ -adrenoceptor blocking agent, dichloroisoprenaline (DCI) could antagonise depressant responses to NA in the cerebral cortex of the rat; however, DCI also had a powerful direct depressant effect on the firing rate of the neurones.

In conclusion, the review of the literature suggests that the  $\alpha$ -adrenoceptor blocking agents are effective in antagonising excitatory responses to NA (Johnson et al., 1969a). Both Johnson, et al. (1969a) and Stone (1973a) emphasized the relative inability of these drugs to modify depressant responses to NA. In contrast to these authors, Frederickson et al., (1972) described the antagonism of depressant responses to NA by  $\alpha$ -adrenoceptor blocking agents; this effect, however, probably was not specific, since responses to ACh were also affected. On the other hand, the  $\beta$ -adrenoceptor blocking agents appear to be effective in antagonising both depressant (Johnson et al., 1969a; Stone, 1973a; Freedman et al., 1975) and excitatory (Johnson et al., 1969a; Stone, 1973; Bevan et al., 1974a) responses.

#### b. Drugs interfering with the cyclic-AMP system

Lake et al. (1972) found that the phosphodiesterase inhibitors aminophylline and papaverine could potentiate depressant



responses to NA. These authors emphasized, however, that this does not necessarily imply that the effects of NA are mediated via cyclic-AMP, since the potentiation might have been due to the direct depressant effect of aminophylline and papaverine.

c. Drugs interfering with the transport and binding of calcium

In a series of papers, Phillis and his colleagues examined the hypothesis that calcium may play an important role in the mediation of the depressant responses to NA and to other monoamines. These authors found that drugs which interfere with the transport and binding of calcium (e.g. lanthanum, verapamil, manganese, nickel, cobalt, neomycin, ruthenium) effectively antagonised the depressant responses to the monoamines (NA, 5-HT, dopamine, histamine) and also the depressant responses to ACh, without affecting depressant responses to GABA and excitatory responses to ACh (Phillis et al., 1973; Yarbrough et al., 1974; Phillis, 1974).

d. Other drugs

Phillis & York (1967) reported that strychnine antagonised the depressant effect of NA on cortical neurones. This observation, however, could not be repeated by Stone (1973a; 1973c).

Anderson & Stone (1974) found that depressant responses to NA could occasionally be antagonised by bulbocapnine.

## IV.2. METHODS

### IV.2.1. PREPARATION OF THE ANIMALS

#### a. Cats

See III.2.1.

#### b. Rats

Male albino Wistar rats, weighing between 250g and 300g, were used.

Anaesthesia was induced with halothane (3.0%), delivered from a temperature and flow-rate compensated vapourizer (Fluotec, Mk 3, Cyprane Ltd.). Tracheostomy was then performed and a tracheal cannula connected to a Y-piece inserted for the administration of the anaesthetic gas mixture. Animals respired spontaneously throughout the experiment. During the course of the preparation of the animal, the halothane concentration was gradually reduced, so that by the time the animal was ready for recording the halothane concentration was 0.5% - 1.0%, and was maintained at this level throughout the experiment. The flow-rate of oxygen used was 500 ml/min.

The animal's head was held rigidly in a stereotaxic frame (David Kopf), and an area of the cerebral cortex was exposed for recording (see III.2.1.). The dura was either incised with a hypodermic needle under microscopic control (see III.2.1.), or was penetrated directly with the micropipette. The recording site was defined by the following stereotaxic coordinates:

A 4.8 - 6.5; L 0.9 - 2.4 (König & Klippel, 1963); this area corresponds to the somatosensory cortex.

ECG and rectal temperature were monitored continuously throughout the experiment. Rectal temperature was maintained between 37° and 38°C by a heating pad placed under the belly of the animal.

#### IV.2.2. PREPARATION OF THE MICROPIPETTES

Five- and six-barrelled micropipettes were prepared as described in II.2. and III.2.2.

The following drug solutions were used: noradrenaline bitartrate (0.2 M or 0.05 M, pH 3.0 - 3.5; 5-hydroxytryptamine bimaleate (0.2 M, pH 3.0 - 3.5); mescaline hydrochloride (0.2 M, pH 3.5 - 4.5); acetylcholine chloride (0.2 M, pH 4.0 - 4.5).

In one series of experiments in which the effects of pH were examined (see IV.3.3.), a different method was used for the filling of the micropipettes. Five-barrelled micropipettes were pulled, and their tips broken to the desired diameter (3.0 - 6.0  $\mu$ ). Freshly prepared drug solutions were then introduced into the barrels, and air was expelled from the tips by centrifugation at 3000 r.p.m. for 30 min (see Curtis, 1964). The solutions used in these experiments were noradrenaline bitartrate (0.2 M, pH 3.1); noradrenaline bitartrate (0.2 M, pH 5.0), and hydrochloric acid (0.01 M, pH 2.0). The solution of noradrenaline bitartrate at pH 3.1 ( $\pm$  0.1) was obtained by dissolving (-)-noradrenaline bitartrate in glass-distilled water, whereas the solution at pH 5.0

( $\pm$  0.1) was obtained by adjustment with 1.0 N NaOH solution. The pH values of these solutions were measured using a Pye Universal pH meter (model EJ 660). Hydrochloric acid solutions of 0.01 M were used because this was the lowest concentration which adequately carried electrophoretic currents.

#### IV.2.3. RECORDING OF ACTION POTENTIALS, MICROELECTROPHORETIC APPLICATION OF DRUGS

See III.2.3. and III.2.4.

### IV.3. RESULTS

#### IV.3.1. RESPONSE TYPES

Three kinds of responses to microelectrophoretically applied NA could be observed: 1. excitatory responses; 2. depressant responses; 3. biphasic responses. The excitatory and depressant responses are monophasic responses which have a latency, an initial rising (or declining) phase, a plateau, and a terminal declining (or rising) phase (see III.1.3.). The biphasic responses invariably consist of an initial depressant phase, followed by an excitatory phase. On no occasion could we observe the two phases in the reverse order, i.e. an excitation was never followed by a depression. The same three types of responses could be observed not only with NA, but also with other monoamines (e.g. 5-HT and mescaline; see Fig. 1/IV). The same response types could be observed both in cats and rats.

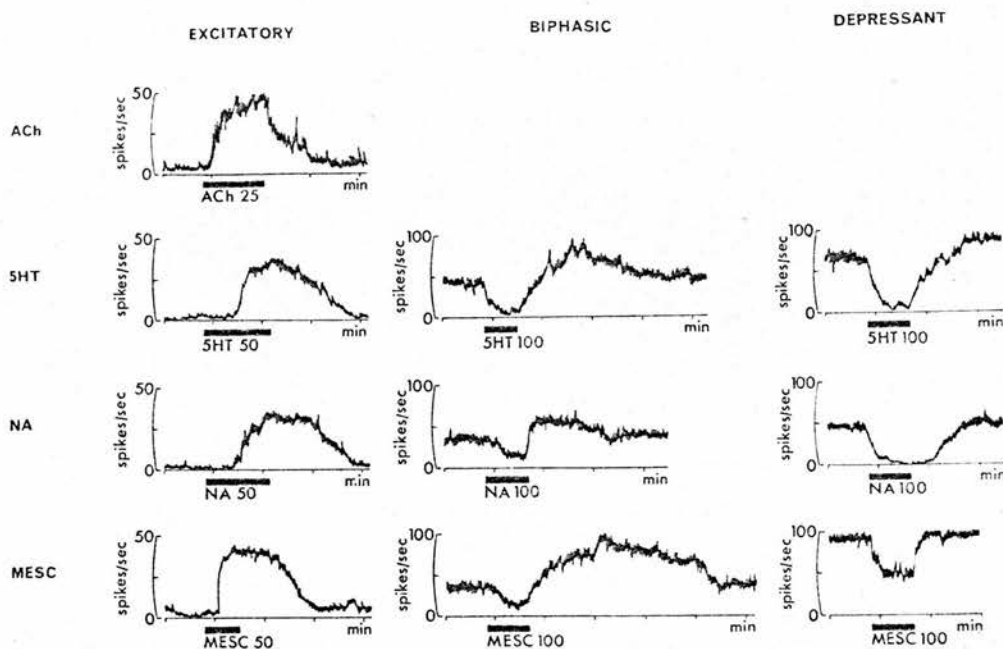


FIGURE 1/IV. Examples of the types of neuronal responses to monoamines and acetylcholine observed in the cerebral cortex of the cat.

Each excerpt is taken from ratemeter recordings of the firing rate of an individual cortical neurone. Ordinates: firing rate (spikes/sec); abscissae: time (min). Horizontal bars indicate drug applications (numbers refer to intensity of ejecting current (nA)).

Acetylcholine (ACh) was excitatory, whereas noradrenaline (NA), 5-hydroxytryptamine (5-HT), and mescaline (MESC) could evoke excitatory, depressant and biphasic responses.

TABLE 1/IV. Responses to noradrenaline

	Number of cells responding			
	Excitation	Depression	Biphasic	Total
Cat	388 (76.4%)	108 (21.3%)	12 (2.3%)	508 (100%)
Rat	338 (70.3%)	139 (28.9%)	4 (0.8%)	481 (100%)

The proportions of cells responding in each of the three possible ways are shown in Table 1/IV. It is apparent from the table, that there was a predominance of excitatory responses in both species: about three quarters (76.4% in the cat, and 70.3% in the rat) of all the responses were excitations, and only about one quarter (21.3% in the cat, and 28.9% in the rat) of the responses were depressions. The proportions of cells responding in a biphasic fashion were small in both species (2.3% and 0.8%). The predominance of excitatory responses was highly significant in both species (binomial test;  $p < 0.0001$ ). There was a small, but statistically significant difference between the frequencies of

occurrence of excitations and depressions in the two species: in the cat, excitations were slightly more common, and depressions slightly less common than in the rat ( $\chi^2$  test;  $p < 0.01$ ).

#### IV.3.2. THE ROLE OF THE BASELINE FIRING RATE

##### a. Cat

We have examined the relationship between the baseline firing rates of the neurones and the direction of responses to NA.

For this analysis, cells were selected to which NA was applied with an ejecting current of +50 nA. For the sake of comparison, responses to 5-HT and mescaline were also analysed; the current to apply 5-HT was +50 nA, and the current to apply mescaline was +100 nA. Each drug was applied at least on two occasions to each cell. Only cells showing consistent responsiveness or lack of responsiveness were included in the statistical analysis.

The effects of NA were studied on 325 cells: 272 cells (83.7%) gave consistent responses, and 53 cells (16.3%) did not respond. The total population of 325 cells was divided into seven groups on the basis of the firing rate (spikes/sec): 0 - 5; 6 - 10; 11 - 15; 16 - 20; 21 - 25; 26 - 30; 31 - 50. As the proportion of non-responsive cells did not depend on the firing rate (product moment correlation:  $r = 0.46$ ,  $p > 0.1$ ), we included only the population of cells which did respond to NA (272 neurones) in our further analysis. The proportions of cells excited and depressed

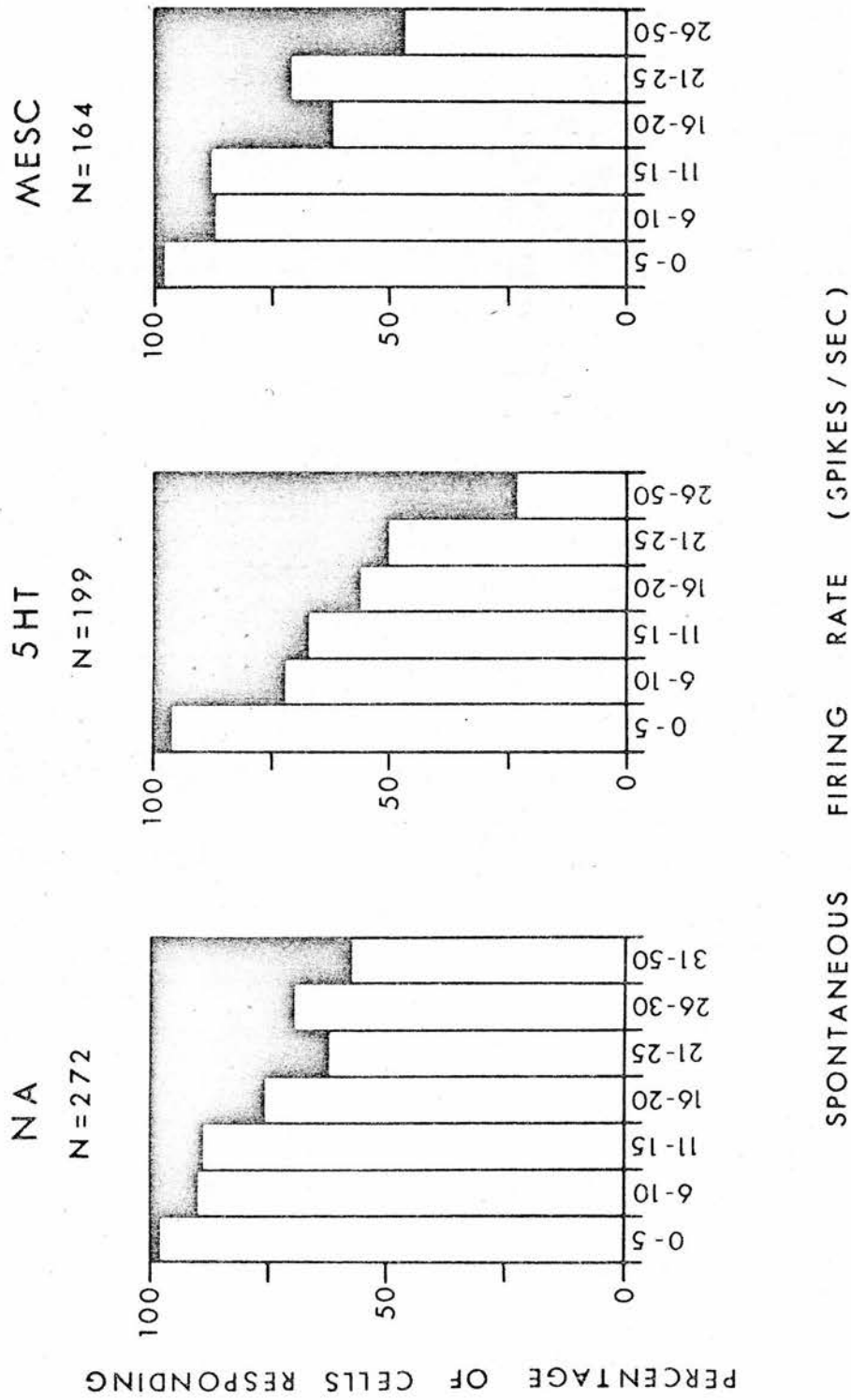
by NA were calculated separately for each firing rate category. The results are shown in Fig. 2/IV: with increasing firing rates the proportion of excitations decreased, and the proportion of depressions increased (product moment correlation:  $r = 0.95$ ,  $p < 0.001$ ).

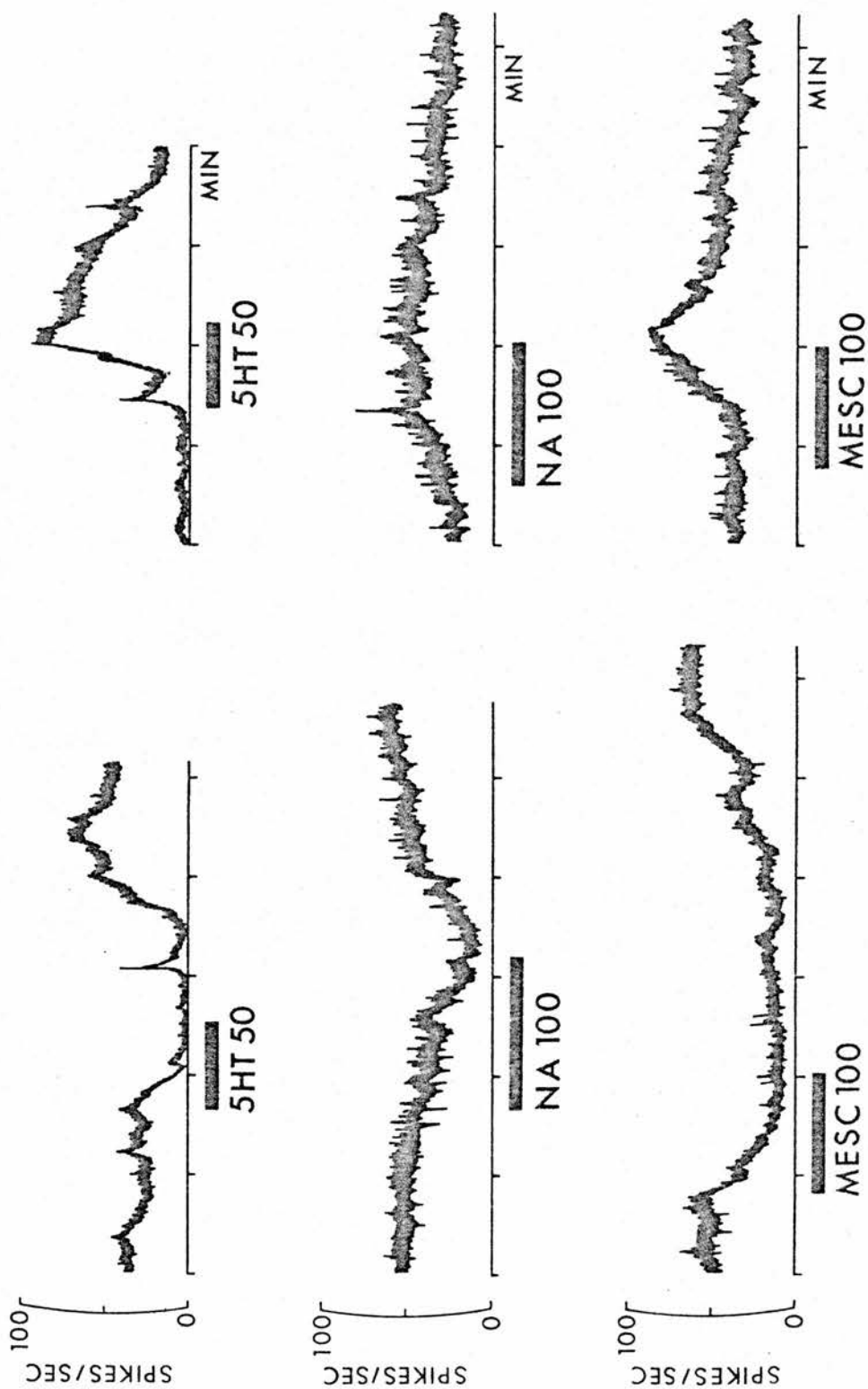
Similar results were obtained with 5-HT and mescaline. Out of 252 cells, 199 cells (79%) responded to 5-HT, and out of 189 cells, 164 (86%) responded to mescaline. In the case of both 5-HT and mescaline, the proportions of cells excited decreased, and the proportions of cells depressed increased with increasing firing rates (5-HT:  $r = 0.98$ ,  $p < 0.001$ ; mescaline:  $r = 0.94$ ,  $p < 0.005$ ) (Fig. 2/IV).

On a few cells, which were not included in the statistical analysis described above, we observed that a spontaneous shift in the baseline firing rate was accompanied by a reversal of the direction of the response to NA, 5-HT or mescaline. On each occasion, a lower firing rate corresponded to an excitatory response, and a higher firing rate to a depressant response. Examples of these observations are shown in Fig. 3/IV.

After having established a close correlation between firing rate and direction of response to the monoamines, we also examined whether there was any relationship between the anatomical position of a neurone in the cortex and the firing rate recorded. To this end, we selected randomly the results from 20 cats out of the total experimental material collected from 257 animals. All the cells recorded in these 20 cats were displayed in the







graph shown in Fig. 4/IV. In this graph, each dot represents a cell, and the position of any individual neurone is determined by the depth in the cortex and the spontaneous firing rate. The figure shows that there was no statistically significant correlation between the location of the cell within the cortex and the firing rate recorded (product moment correlation;  $r = 0.007$ ,  $p > 0.2$ ).

#### b. Rat

For this analysis, 332 cells were selected which gave consistent excitatory or depressant responses to NA applied with an ejecting current of +25 nA. The same procedure was adopted as with the data from the cat (see above); the proportions of cells excited and depressed were calculated for each of the seven firing rate categories. The results are shown in Fig. 5/IV: with increasing firing rates the proportion of excitations decreased, and the proportion of depressions increased (product moment correlation:  $r = 0.84$ ,  $p < 0.02$ ).

On a few cells, which were not included in the statistical analysis, we observed that a shift in the baseline firing rate was accompanied by a reversal of the direction of the response to NA. On each occasion, a lower firing rate corresponded to an excitatory response, and a higher firing rate to a depressant response. An example of this observation is shown in Fig. 6/IV.

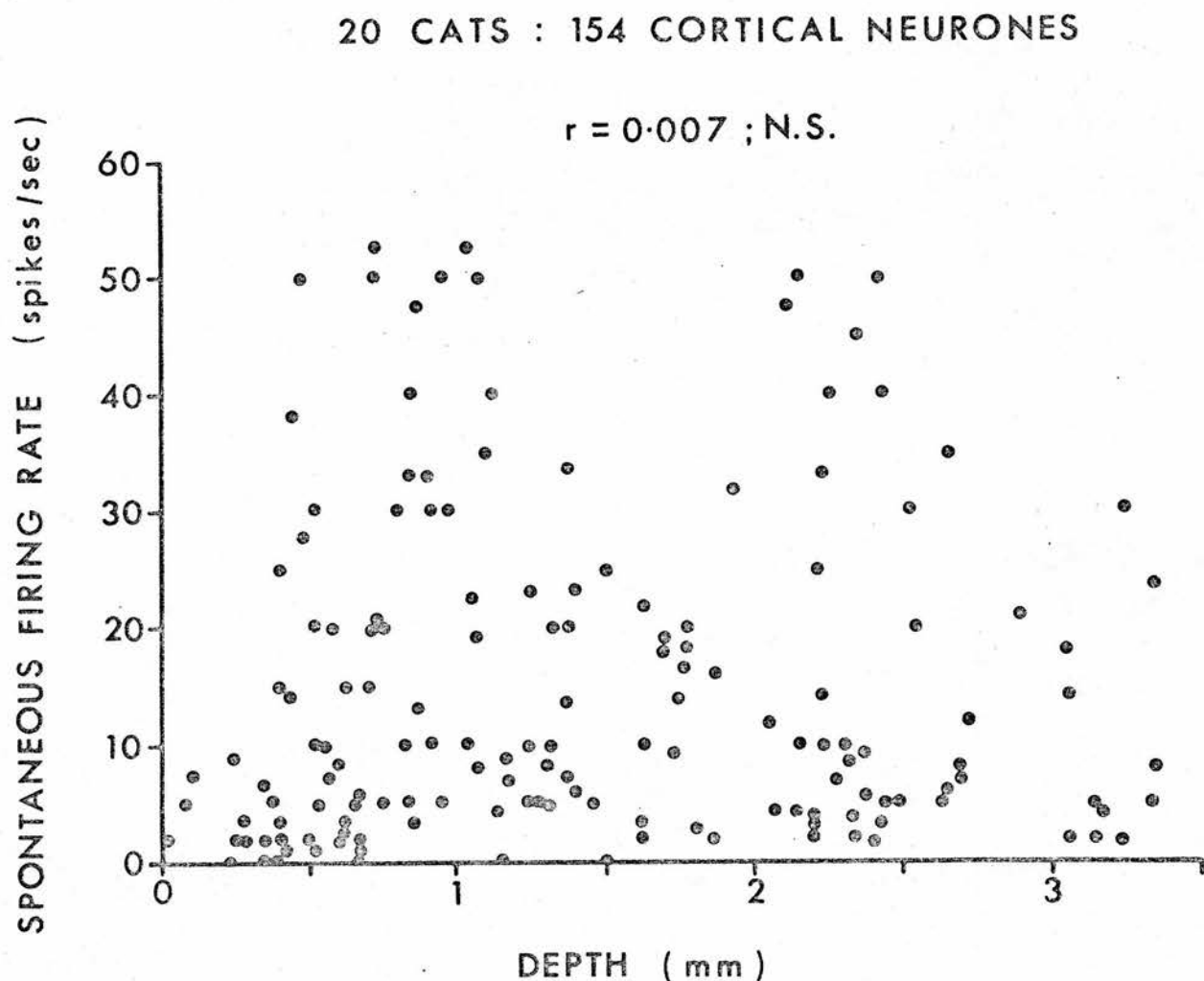


FIGURE 4/IV. Relationship between the positions of neurones in the somatosensory cortex of the cat and their spontaneous firing rate.

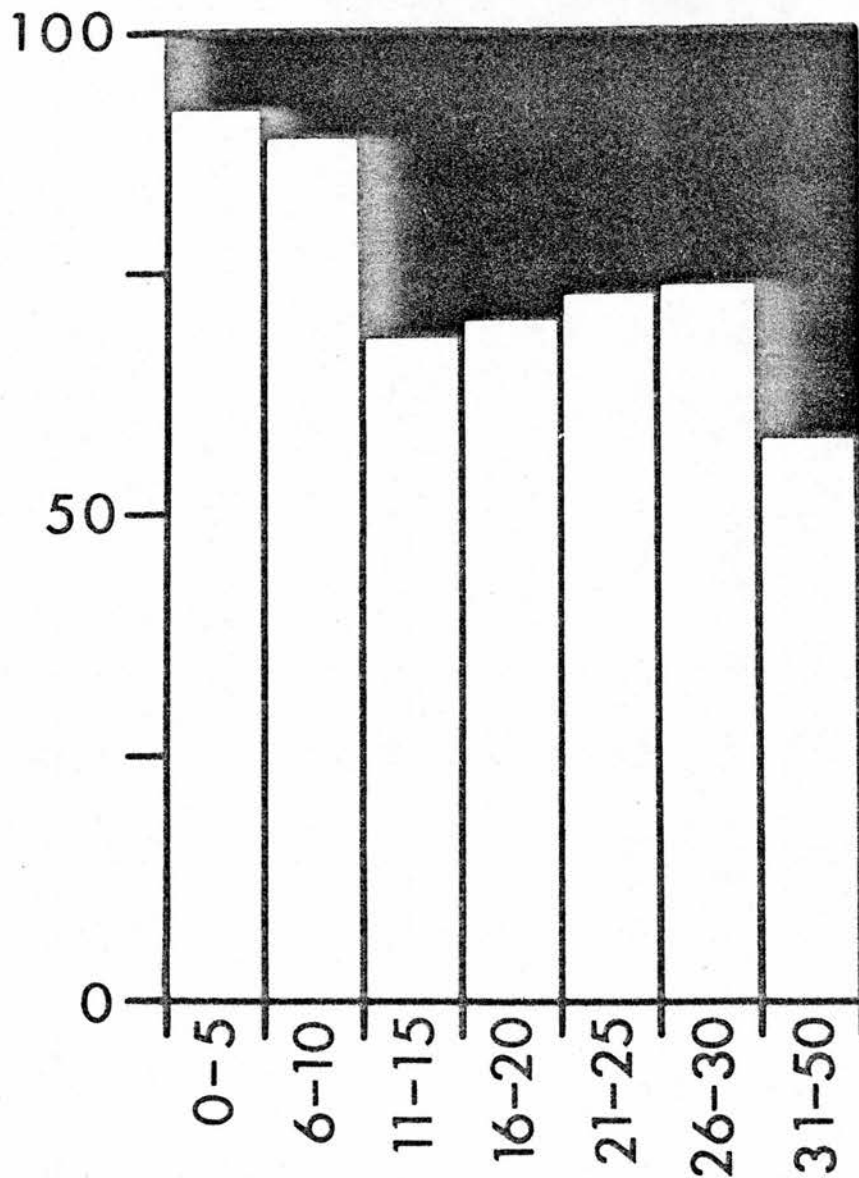
Data were collected from 154 neurones recorded in 20 cats. Ordinate: spontaneous firing rate (spikes/sec); abscissa: depth from the surface of the cortex (mm). Each dot corresponds to a neurone.

There is no correlation between depth in the cortex and spontaneous firing rate ( $r = 0.007$ ,  $P > 0.2$  (N.S.)).

# RAT CORTEX

NA 25nA ; n = 332

PERCENTAGE OF CELLS RESPONDING



SPONTANEOUS FIRING RATE  
SPIKES/SEC

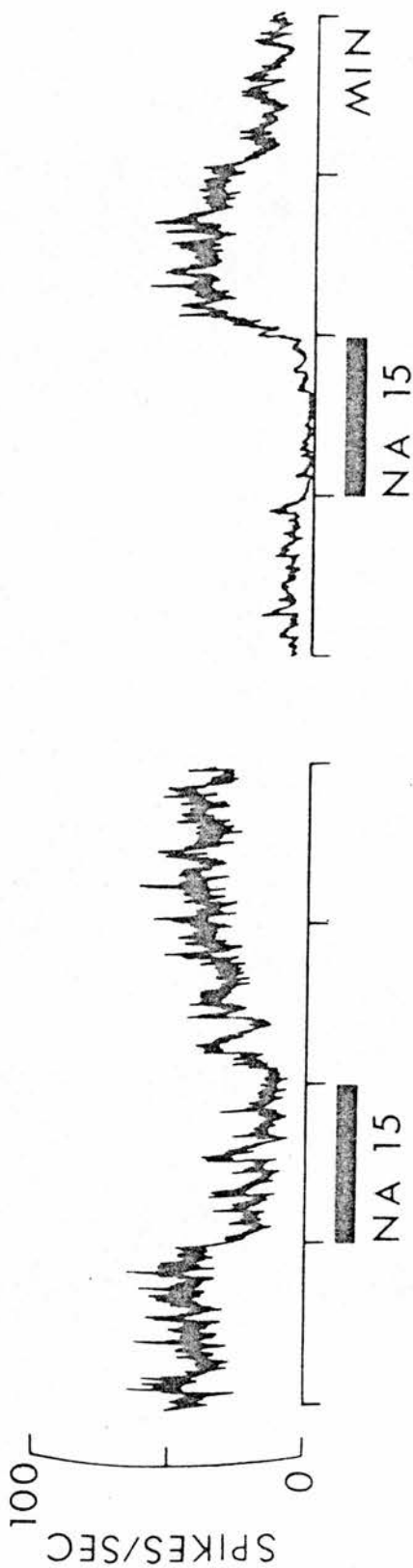


FIGURE 6/IV. Spontaneous reversal of the direction of the neuronal response to noradrenaline following a shift in the baseline firing rate.  
 Excerpts from the ratemeter recording of the firing rate of a single cortical neurone in the rat. Ordinate: firing rate (spikes/sec); abscissa: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensity of ejecting current (nA).  
 The cell was depressed by noradrenaline (NA) when the baseline firing rate was high, and excited by noradrenaline when the firing rate was low.

### c. Comparison of the two species

Table 1/IV shows that excitations to NA were slightly more common, and depressions were slightly less common in the cat than in the rat; this difference between the two species was statistically significant (see IV.3.1.). Therefore, it was of interest to examine whether this difference in the proportions of cells excited or depressed could reflect a difference in the baseline firing rates of the neurones recorded in the two species.

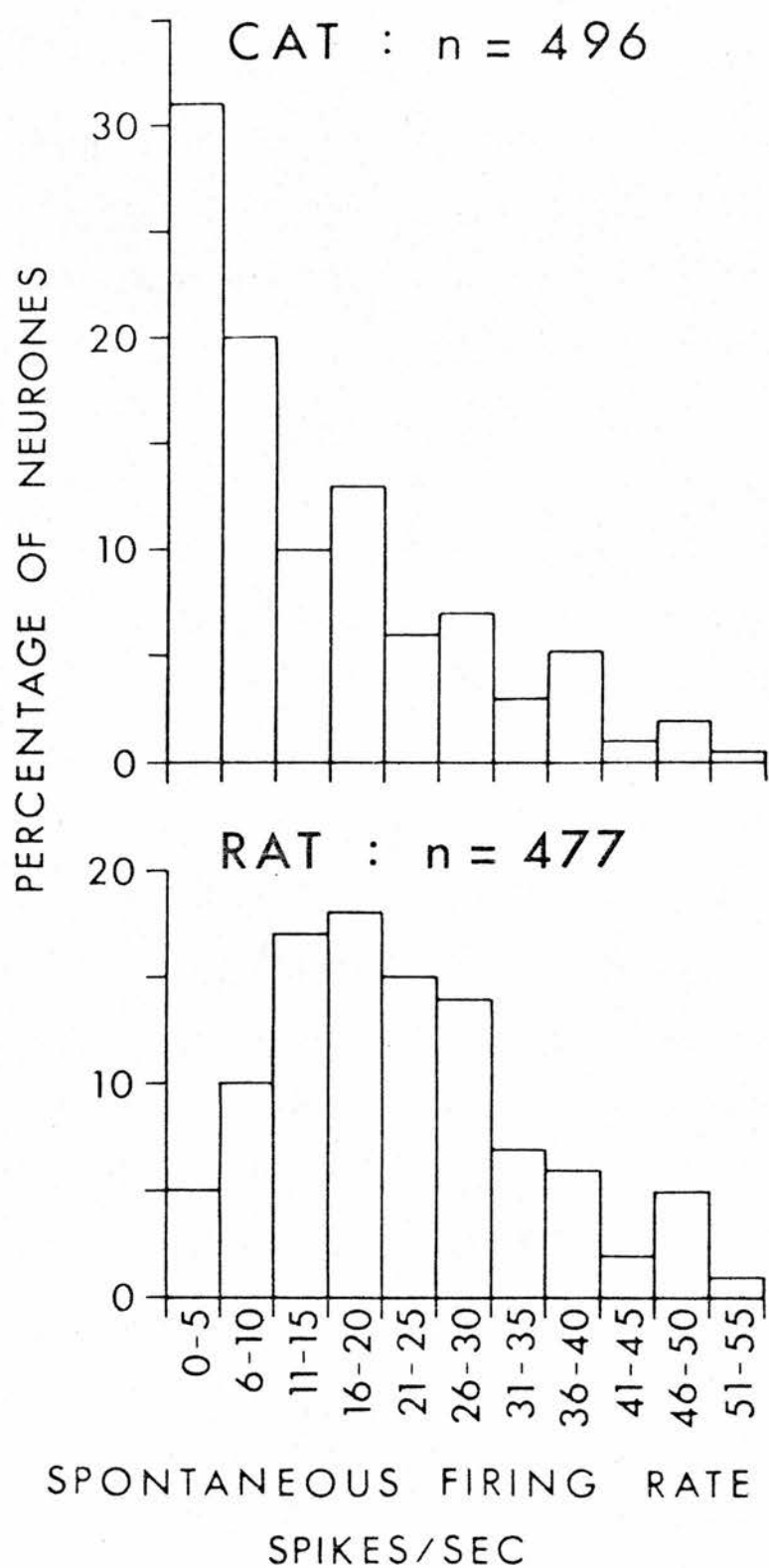
Fig. 7/IV shows the distribution of the neurones, included in Table 1/IV, between eleven firing rate categories. It is apparent from the figure that the frequency distribution of firing rates was different in the two species: the distribution obtained in the cat having a greater skew to the left than the distribution obtained in the rat. This is reflected in a lower median firing rate in the cat (10 spikes/sec) than in the rat (20 spikes/sec). The difference between the two patterns of distribution is statistically significant (median test;  $p < 0.001$ ).

### IV.3.3. THE ROLE OF THE pH OF THE NORADRENALINE SOLUTION

These experiments were conducted in cats. The responses of 16 neurones to NA released from solutions at pH 3.1 and 5.0 and to  $H^+$  released from a HCl solution were compared.

#### a. Comparison of NA (pH 3.1) and NA (pH 5.0)

Of the 16 cells studied, the 13 neurones which were excited by NA (pH 3.1) were also excited by NA (5.0), and the





3 neurones depressed by NA (pH 3.1) were also depressed by NA (pH 5.0). No cell responded differently to the drug at different pH values. An example of this finding is shown in Fig. 8/IV.

#### b. Effect of $H^+$

Of the 16 NA-sensitive cells, 15 did not respond to  $H^+$  even when currents of up to +200 nA were used (see Figs. 8/IV and 10/IV). One neurone, which was depressed by NA, was also depressed by  $H^+$ . Changes in spike amplitude did not occur.

The application of  $H^+$  frequently reduced the size of the response to a subsequent application of NA. Furthermore, when NA (pH 5.0) and  $H^+$  were ejected simultaneously from adjacent barrels of the micropipette, there was a reduction in the size of the excitatory response (Fig. 9/IV).

#### c. Dose-response studies

It was observed on 9 cells that NA (pH 3.1) evoked larger responses than NA (pH 5.0) (see Fig. 8/IV). As this suggested a difference in potency between NA (pH 3.1) and NA (pH 5.0), we conducted detailed comparative studies on three cells. A range of current intensities was used to compare the responses of the same cell to NA (pH 3.1) and NA (pH 5.0). The results obtained from one cell are displayed in Fig. 10/IV and 11/IV. At the lower current intensities NA (pH 3.1) and NA (pH 5.0) were approximately equipotent, whereas NA (pH 3.1) was considerably more potent when currents of higher intensities were used.

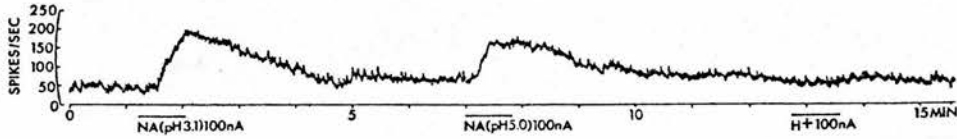


FIGURE 8/IV. Responses of a single cortical neurone to noradrenaline (NA) ejected from solutions at different pH.

Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents (nA)).

The neurone was excited by NA (pH 3.1) and pH (5.0), but did not respond to  $H^+$ .

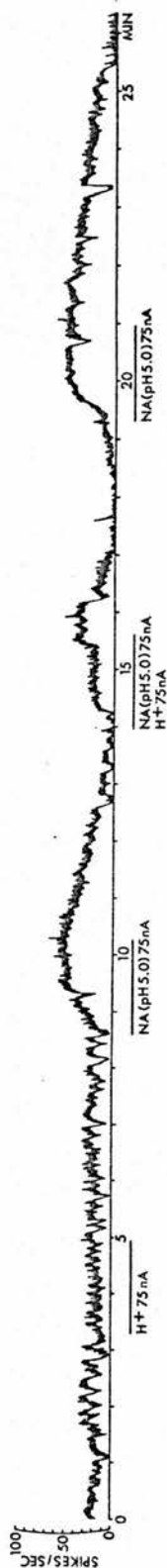


FIGURE 9/IV. Effect of  $H^+$  on excitatory responses of a cortical neurone to noradrenaline (NA). Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting currents (nA)).  
The size of the excitatory response to NA (pH 5.0) was reduced when it and  $H^+$  were applied simultaneously.

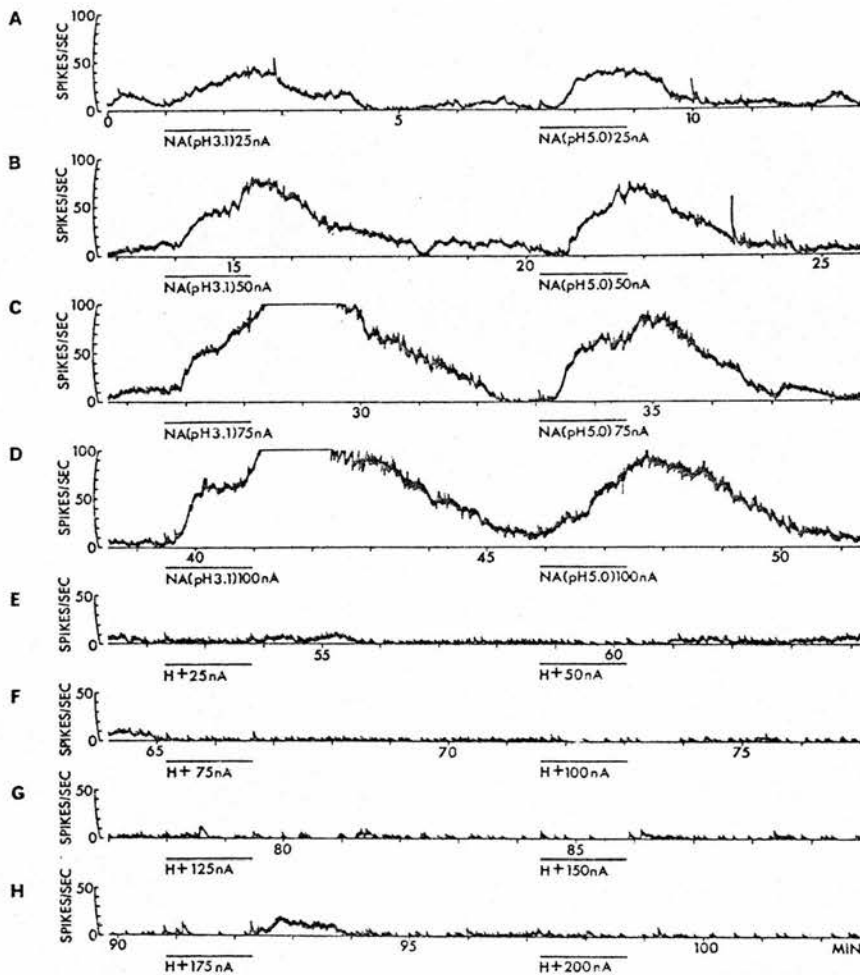


FIGURE 10/IV. Excitatory responses of a cortical neurone to noradrenaline applied from solutions at different pH.

Continuous recording of the firing rate of a single cortical neurone. Ordinate: firing rate (spikes/sec); abscissa: time (min). Horizontal bars indicate drug applications (numbers refer to intensities of ejecting current (nA)).

A - D: NA (pH 3.1) and NA (pH 5.0) applied with successively higher currents evoked progressively larger responses. At higher current intensities NA (pH 3.1) evoked larger responses than NA (pH 5.0).

E - H: H<sup>+</sup> (25 - 200 nA) failed to evoke responses.

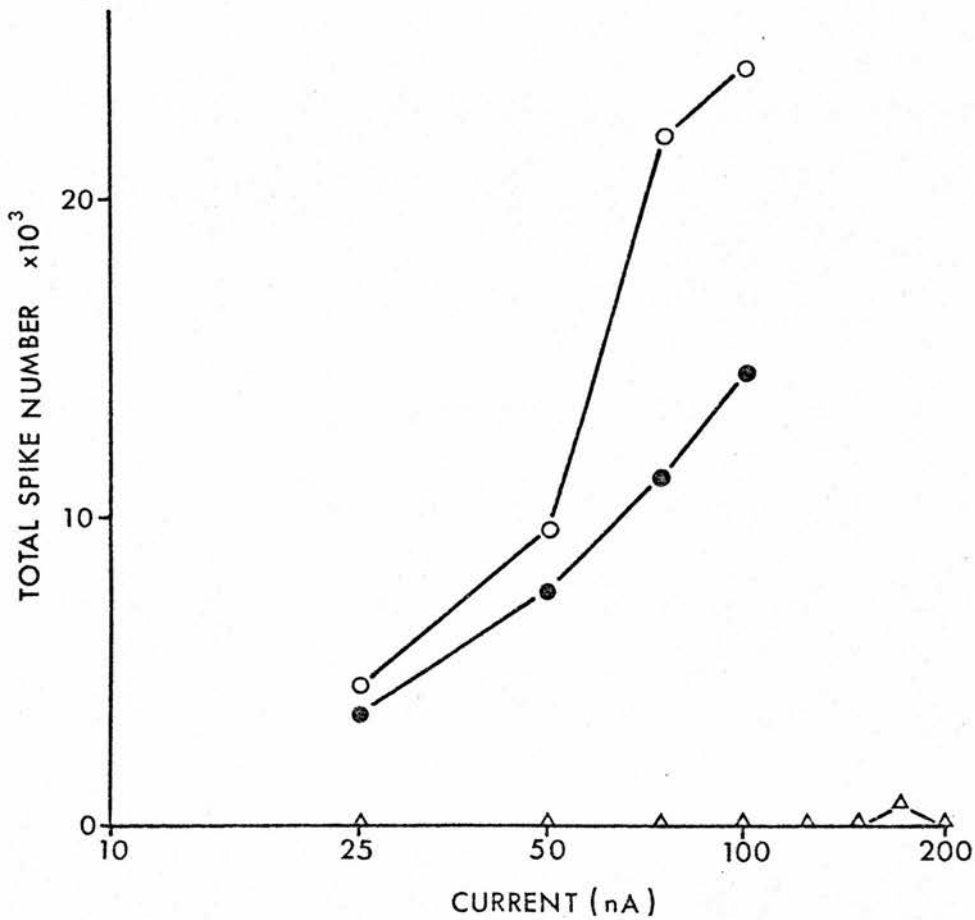


FIGURE 11/IV. Magnitude of excitatory responses of a cortical neurone to noradrenaline (NA) applied from solutions at different pH.

Ordinate: total spike number; abscissa: intensity of ejecting current (nA), on a log scale. Open circles: NA (pH 3.1); closed circles: NA (pH 5.0); open triangles: H<sup>+</sup>. Data are derived from the study shown in Fig. 7/IV.

NA (pH 3.1) was more potent than NA (pH 5.0) at higher ejecting current intensities.

#### IV.4. DISCUSSION

##### IV.4.1. THE DUAL EFFECT OF NORADRENALINE ON CORTICAL NEURONES

In agreement with previous findings (see IV.1.1.), we could observe both excitatory and depressant neuronal responses to microelectrophoretically applied NA in the neocortices of both cats and rats. In our material there was a predominance of excitatory responses: about three quarters of all responses were excitatory in both species studied. Similar results were reported by Johnson et al., (1969a; 1969b) in the halothane-anaesthetised cat.

Although most authors have described both excitatory and depressant responses to NA on cortical neurones (see IV.1.1.), there has been some controversy concerning the true nature of these responses. One group of authors suggested that the depressant responses represented the genuine pharmacological effect of NA, whereas the excitatory responses were either artefacts (Krnjević & Phillis, 1963c; Frederickson et al., 1971) or indirect effects (Stone, 1971). Another group of investigators argued that the excitatory responses were genuine, whereas the depressant responses might be 'non-specific' in nature (Johnson et al., 1969a).

On the basis of our results, there is no a priori reason for assuming that only one kind of neuronal response represents a genuine pharmacological action of NA, whereas the other kind of response is artefactual. Indeed, this thesis presents experimental evidence which suggests that both the excitatory and

depressant responses are genuine pharmacological effects, in other words, that NA can activate both excitatory and inhibitory receptors on cortical neurones.

This chapter already presents some evidence which suggests that both excitatory and inhibitory receptors to NA may occur on the same neurone. The existence of biphasic responses could be regarded as one piece of such evidence. Biphasic responses are not unique to the cortex: such responses to NA have been described in the spinal cord (Weight & Salmoiraghi, 1966), Deiters' nucleus (Yamamoto, 1967), medial geniculate nucleus (TebŒcis, 1970), and brain stem (Boakes et al., 1971). The time-course of the biphasic response suggests that inhibitory receptors may be more sensitive to NA, since the depressant phase appears first when the concentration of the drug at receptor sites is probably still rising. The excitatory phase may reflect the presence of a higher drug concentration which is necessary for the activation of the less sensitive excitatory receptors. Observations on invertebrate ganglion cells (see Gerschenfeld, 1973) have shown that both excitatory and inhibitory receptors to the same transmitter substance can co-exist on the same neurone. Moreover, it has been shown on invertebrate neurones that the activation of two functionally opposing receptor populations can be the basis for biphasic responses (Ascher, 1972).

An alternative explanation for biphasic responses could be that there is only one type of receptor (e.g. excitatory) which is located on a principal neurone and on an inhibitory interneurone. The first, depressant phase of the response would reflect the indirect, whereas the second excitatory phase the direct activation of the principal neurone. However, if an action on an interneurone were responsible for one of the phases, it might be expected that the sequence of occurrence of the two phases would occasionally be reversed, depending on the relative positive positions of the tip of the micropipette, the interneurone, and the recorded neurone. It has been observed, however, that the depressant phase always precedes the excitatory one (Boakes et al., 1971; see also present Results). Furthermore, if inhibitory interneurons were responsible for biphasic responses to the monoamines, it is surprising that there is no direct evidence concerning the existence of such a mechanism. It has been observed in the olfactory bulb (McLennan, 1971) and in the dorsal horn of the spinal cord (Zieglgänsberger & Herz, 1971) that an 'excitant' amino acid can cause paradoxical depression of the principal neurone accompanied by the excitation of a smaller neurone recorded simultaneously. On cortical neurones, however, we have never observed excitation of a smaller neurone while recording the depressant phase of a biphasic response to NA or to another monoamine.



The phenomenon of response reversal (Fig. 3/IV and 6/IV) is further evidence in favour of the hypothesis that both excitatory and inhibitory receptors for NA can occur on the same neurone. This observation would suggest that even in the case of monophasic responses both excitatory and inhibitory receptors may be activated, and the time-course of the response reflects the relationship between the two receptor populations. This has been demonstrated to occur on invertebrate ganglion cells (Kehoe, 1972b). In this case, one type of receptor is dominant (i.e. its activation determines the direction of the observed response), whereas the opposite type of receptor is masked for the whole time-course of the response. In the case of response reversal a switch in receptor dominance may occur: when the baseline is low, the excitatory receptors are dominant, and when the baseline shifts to a higher position, the inhibitory receptors become dominant.

Further evidence in favour of the co-existence of functionally opposite NA receptors on the same neurones will be presented in Chapter V.

In conclusion, the results presented in IV.3. are consistent with the hypothesis that NA can activate both excitatory and inhibitory receptors on cortical neurones. This effect of NA is not surprising, since a similar dual action is one of the most striking properties of this drug in peripheral tissues (see e.g. Furchgott, 1972). Moreover, there is evidence indicating that in most other structures of the brain (e.g. spinal cord, brain stem, hypothalamus) NA has a similar dual effect (see I.4.).

#### IV.4.2. FACTORS MODIFYING THE DIRECTION OF THE RESPONSE TO NORADRENALINE

##### a. Baseline firing rate

The data presented in IV.3.2. show that the probability of observing an excitatory response to NA (and to the other monoamines) depends on the firing rate of the neurone: a greater proportion of cortical neurones is excited when the firing rate is low. The same correlation was found both in the cat and the rat. Our results are consistent with the hypothesis that most cortical neurones contain both excitatory and inhibitory receptors for the monoamines, and that neuronal responses to the monoamines reflect a 'functional balance' between the two kinds of receptor (see IV.4.1.). The data presented here suggest that the relationship between excitatory and inhibitory receptors may be influenced by the baseline firing rate of the neurone: a low firing rate would favour the functional dominance of the excitatory receptors, whereas a high firing rate would shift the balance in favour of the inhibitory receptors.

An alternative possibility is that the slowly and fast firing cells represent two anatomically distinct populations of cortical neurones: the slow cells contain mainly excitatory receptors, whereas the fast cells possess mostly inhibitory receptors. This hypothesis, however, is not supported by the survey shown in Fig. 4/IV: no correlation could be found between location of a neurone in the cortex and its firing rate. Moreover,

the phenomenon of response reversal would favour the hypothesis of the co-existence of the two kinds of receptors on the same cells.

The finding that a spontaneous shift in the baseline firing rate is sometimes accompanied by a reversal of the direction of the response to NA, 5-HT or mescaline (see Fig. 3/IV and 7/IV) seems to be in agreement with observations in the periphery. There is evidence that in smooth muscle preparations containing both excitatory  $\alpha$ - and inhibitory  $\beta$ -adrenoceptors, the direction of the response to NA and adrenaline depends on the baseline activity of the test system. When the baseline tone is low, these drugs evoke contractile responses, whereas the same drugs have a relaxing effect when the baseline tone of the preparation has been raised (Guimarães, 1969; Haffner, 1971).

The relationship between baseline firing rate and direction of the response may explain a difference between the two species: in the cat, where the median firing rate of cortical neurones was lower (10 spikes/sec), excitatory responses to NA occurred significantly more frequently than in the rat, where the median firing rate of cortical neurones was higher (20 spikes/sec) (see Table 1/IV and Fig. 7/IV).

Our observations may also help to explain the discrepancies between reports from different laboratories concerning the

proportions of excitations and depressions to NA (see IV.1.1.). For example, in our material collected from cats (see Table 1/IV), of the 508 cells responding to NA, 76.4% were excited, and 21.3% were depressed. The relatively high proportion of excitatory responses observed by us may reflect the large number of slowly firing cells in our material: about 50% of the neurones had a lower firing rate than 10 spikes/sec, and 73% fired more slowly than 20 spikes/sec. Moreover, it is not surprising that a higher proportion of cells is depressed by NA when the baseline firing rates of the neurones are artificially raised with excitant amino acids (see e.g. Frederickson et al., 1972).

#### b. Role of the pH of the noradrenaline solution

The present results (IV.3.3.) confirm the observations of Johnson et al., (1969a) and Stone (1972) that NA ejected from solutions at high and low pH values has qualitatively the same effect. Thus it is unlikely that the occurrence of excitatory responses to NA is due to its use in solutions at low pH values. This conclusion is further supported by the frequent observation of excitatory responses of brain stem neurones to NA applied from solutions at pH 5.0 - 6.0 (Boakes, et al., 1971). Furthermore, phenylephrine appeared to be exclusively excitatory on cortical neurones (see Chapter V), and this drug was released from a solution at pH 5.0 - 5.5.

We were unable to confirm the findings of Jordan et al. (1972b) and Hewes & Frederickson (1974) that cortical

neurones can be excited by  $H^+$  ejected from HCl solutions. Indeed,  $H^+$  reduced the size of excitatory responses to NA when  $H^+$  and NA were ejected simultaneously from adjacent barrels of the micropipette.

Although NA released from solutions at different pH values evoked qualitatively similar responses, the size of the depressant or excitatory responses of any neurone to NA ejected from a solution at pH 3.1 was usually greater than that of the response to NA ejected from a solution at pH 5.0. The current-response studies show that when ejected from solutions at pH 3.1, NA appeared to be more potent than when ejected from solutions at pH 5.0, especially when electrophoretic currents of higher intensities were used. The difference between the action of NA released from solutions at different pH values was interpreted by Frederickson et al. (1971) in terms of an excitatory action of  $H^+$  released from solutions at lower pH values. Such an explanation is no longer tenable in the light of the present results. However, the apparent difference in potency may result from a lower rate of NA release from the solution at pH 5.0. This would be due to the large number of  $Na^+$  introduced into the solution when the pH was adjusted to 5.0 (0.1 M in the final solution). This would tend to reduce the transport number of NA. The in vitro experiments described in II.3.5. show that this is indeed the case.

It is apparent, therefore, that there are no good reasons for avoiding the use of NA solutions at pH 3.0 - 3.5 in microelectrophoresis experiments. However, there are disadvantages associated

with the adjustment of the pH to a higher level by the addition of NaOH: firstly, dose-response relations are distorted, and secondly, there is a greater risk of obtaining misleading results due to the application of a biologically active product of oxidation.

C H A P T E R   V

MODIFICATION OF NEURONAL RESPONSES TO NORADRENALINE BY  
ADRENERGIC ANTAGONISTS

### V.1. INTRODUCTION

The evidence presented in the previous Chapter, together with the literature reviewed (IV.1.1.), indicates that single cortical neurones are sensitive to NA applied by microelectrophoresis: both excitatory and depressant responses can be observed. So far, however, these responses have not been characterized in terms of the classical  $\alpha$  and  $\beta$  categories (Ahlquist, 1948).

There are basically three methods available for receptor categorization: comparison of agonists; comparison of antagonists; and desensitization (Schild, 1973). Previous attempts to classify neuronal responses concentrated mainly on the use of antagonists (see IV.1.5.). It has been reported that both  $\alpha$ - and  $\beta$ -receptor blocking agents can antagonise excitatory responses to NA, whereas depressant responses to NA seem to be much more resistant to these antagonists (Johnson et al., 1969a). Johnson et al. (1969a) concluded that the excitatory responses might be mediated by receptors which are different from the classical peripheral  $\alpha$ - and  $\beta$ -adrenoceptors, and that the depressant responses might be 'non-specific' in nature. Stone (1973a) confirmed the relative ineffectiveness of  $\alpha$ -adrenoceptor blocking agents in antagonising depressant responses to NA; he reported, however, that  $\beta$ -adrenoceptor blocking agents could antagonise these responses. Stone (1973a) concluded that the depressant responses might be mediated via  $\beta$ -adrenoceptors.

There are fewer data concerning the effects of different adrenergic agonists. Although it has been reported that isoprenaline (IPNA) can evoke both excitatory and depressant responses on cortical



neurones (Johnson et al., 1969a; Stone, 1973a), the effects of more selective  $\beta$ -adrenoceptor stimulants (e.g. salbutamol), or the effects of more selective  $\alpha$ -receptor stimulants (e.g. phenylephrine, methoxamine) have never been tested.

In the experiments presented in this Chapter, we have compared the effects of a range of  $\alpha$ - and  $\beta$ -receptor stimulating agents on cortical neurones. We have also re-examined the selectivity of adrenergic antagonists.

## V.2. METHODS

All the experiments presented in this Chapter were conducted in rats; for the preparation of the animals see IV.2.1. Our methods for the manufacture of six-barrelled micropipettes, for the extra-cellular recording of action potentials and the microelectrophoretic application of drugs have been described in III.2.

The following drug solutions were used in the micropipettes: (-)noradrenaline bitartrate (0.05 M, pH 3.0 - 3.5); (-)phenylephrine hydrochloride (0.05 M, pH 5.0 - 5.5); (+)methoxamine hydrochloride (0.05 M, pH 4.5); salbutamol sulphate (0.05 M, pH 5.5); (+)isoprenaline hydrochloride (0.05 M, pH 5.5); acetylcholine chloride (0.05 M, pH 3.5 - 4.0); phentolamine mesylate (0.01 M, pH 4.5 - 5.5); phenoxybenzamine hydrochloride (0.01 M, pH 3.0); propranolol hydrochloride (0.01 M, pH 4.5 - 5.5); sotalol hydrochloride (0.01 M, pH 4.0 - 5.0).

Only spontaneously active neurones were studied in these experiments. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive drug applications retaining

currents of -10 nA were passed. Retaining currents of -25 nA were used for the antagonists. Intervals between successive applications of the same agonist were kept constant in order to standardize the effects of the retaining current upon drug release during the ejection period (see II.3.3. and III.3.2.). The sizes of the neuronal responses to the agonists were expressed as the total number of action potentials produced in response to each drug application ('total spike number'; see III.2.5.).

The effects of antagonists were evaluated in the following way. When suitable responses to the agonist had been obtained, the antagonist was applied continuously, either by removal of the retaining current (thus allowing the drug to diffuse out from the micropipette), or by the passage of a weak ejecting current (5 - 10 nA), and the time-course of the developing antagonism was followed. If necessary, the intensity of the ejecting current to apply the antagonist was increased until antagonism was observed. After antagonism of the response to the agonist had been established, application of the antagonist was continued until a further response to the control agonist(s) could be observed. Then the application of the antagonist was terminated, and the time-course of recovery was followed. The response to an agonist was regarded as antagonised if there was at least 50% reduction in the total spike number (Bevan et al., 1974a).

### V.3. RESULTS

#### V.3.1. COMPARISON OF ADRENERGIC AGONISTS

The proportions of cells responding with excitation or depression to each adrenergic agonist studied are shown in Table 1/V.

It is apparent from the table that phenylephrine and methoxamine were exclusively excitatory, whereas salbutamol was entirely depressant. On the other hand, NA and IPNA could evoke both excitatory and depressant responses. Examples of cells responding both with excitation and depression to different agonists are shown in Fig. 1/V.

Table 1/V. Percentage of cortical neurones responding either with excitation or depression to adrenergic agonists

	Percentage of cells responding		n
	Excitation	Depression	
Phenylephrine	100	0	78
Methoxamine	100	0	11
Noradrenaline	66	34	194
Isoprenaline	23	77	138
Salbutamol	0	100	16

IPNA could evoke both depressant and excitatory responses in a dose-dependent fashion on the same cell: on 14 cells a lower current of IPNA evoked a depression whereas a higher current evoked an excitation. Examples of this observation are shown in Fig. 2/V. On the basis of this observation, we could predictably evoke depressant responses to IPNA by applying the drug with relatively low ejecting currents ( $< 25$  nA) (see antagonism studies in V.3.2.).

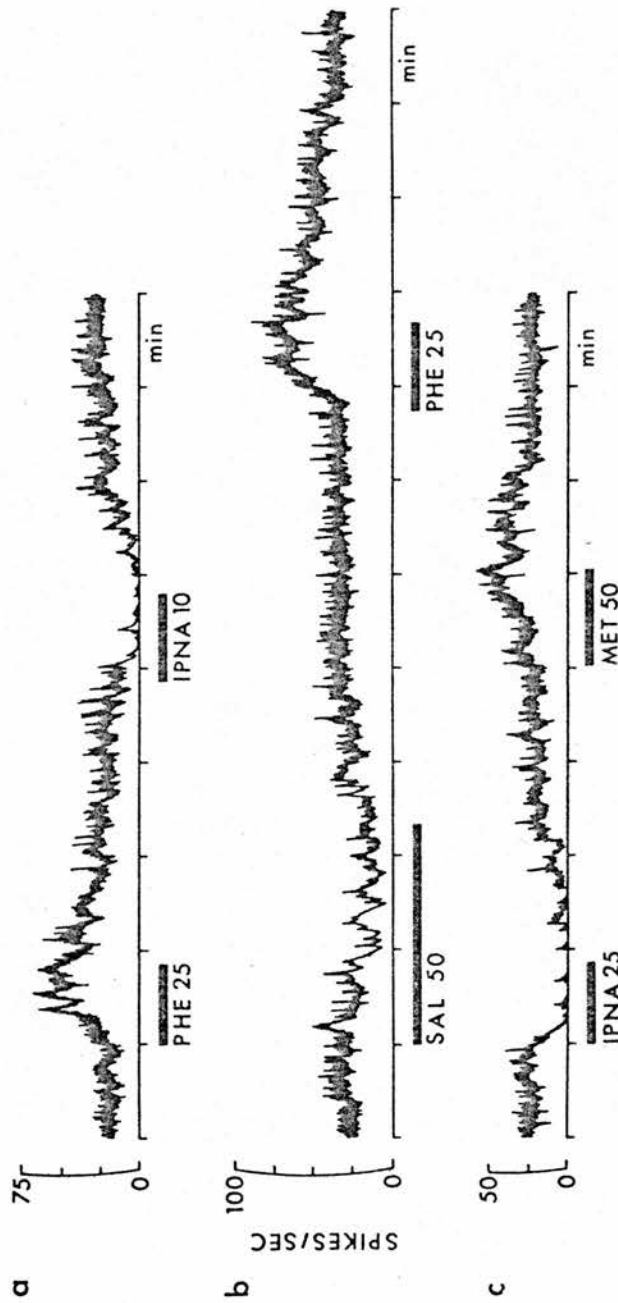


FIGURE 1/V. Examples of the effects of adrenergic agonists on cortical neurones.  
Ratemeter recordings of the firing rates of three cortical neurones in the rat (a, b, c). Ordinates: firing rate (spikes/sec); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents.

- a. A cell excited by phenylephrine (Phe) and depressed by isoprenaline (IPNA).
- b. A cell depressed by salbutamol (Salb) and excited by phenylephrine.
- c. A cell depressed by isoprenaline and excited by methoxamine (Met).

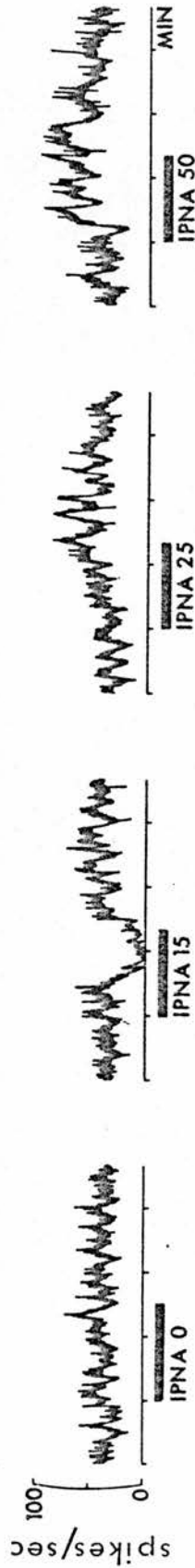


FIGURE 2/V. Responses of a cortical neurone to isoprenaline (IPNA) applied with increasing intensities of ejecting current.

Ratemeter recording of the firing rate of a single cortical neurone in the rat. Ordinate: firing rate (spikes/sec); abscissae: time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents (nA).

Note the reversal of the response from depression to excitation as the intensity of the ejecting current was increased.

The relative potencies of methoxamine and phenylephrine were compared on 10 cells: in order to obtain approximately equivalent responses to the two drugs, the current needed to apply methoxamine was at least five times greater than that needed to apply phenylephrine.

### V.3.2. EFFECTS OF ADRENERGIC ANTAGONISTS

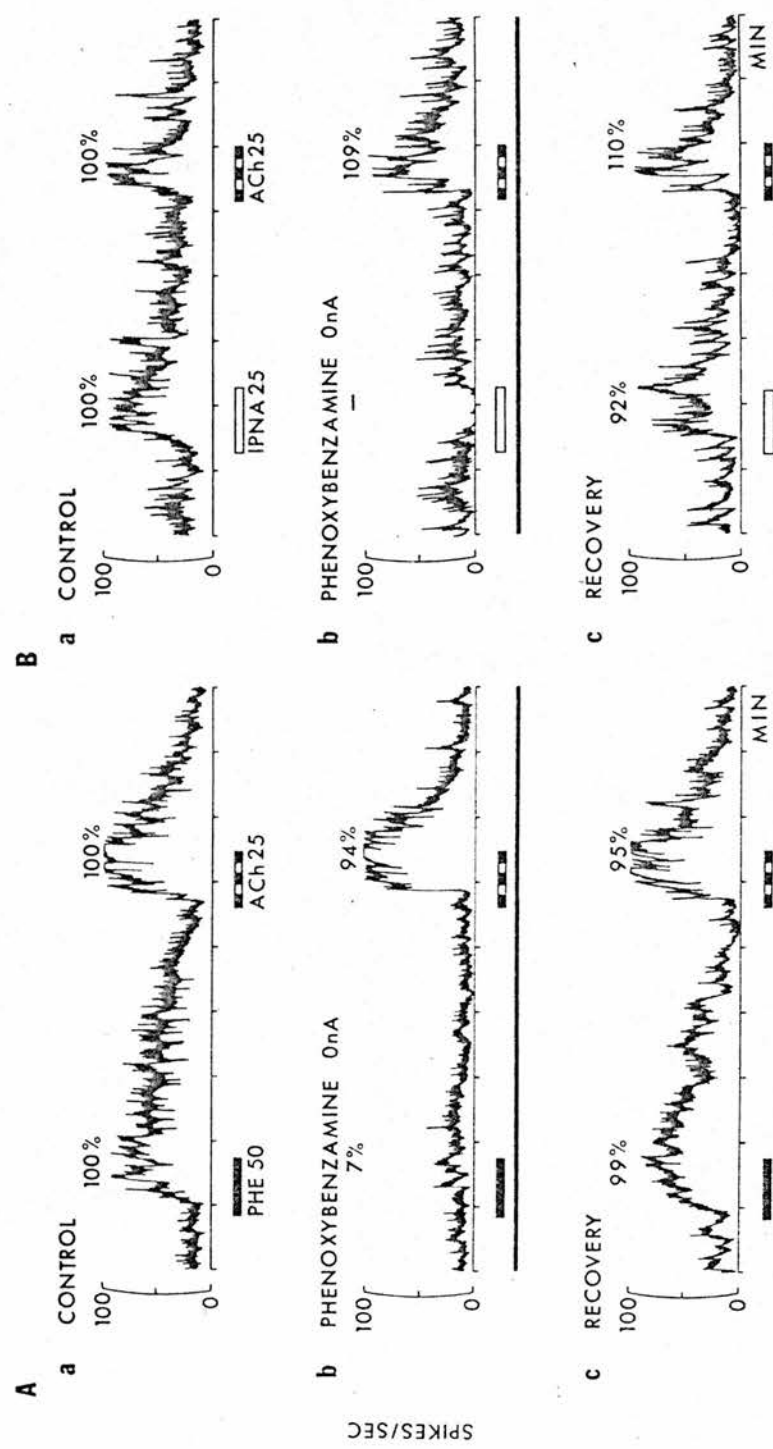
#### a. $\alpha$ -Adrenoceptor blocking agents

The  $\alpha$ -adrenoceptor blocking agents phentolamine and phenoxybenzamine were effective in reversibly antagonising excitatory responses to adrenergic agonists, while excitatory responses to ACh were not affected. Phentolamine reversibly and selectively antagonised excitatory responses to NA on 10 cells.\* Phenoxybenzamine reversibly and selectively antagonised excitatory responses to NA (5 cells), phenylephrine (11 cells), and IPNA (5 cells) (e.g. Fig. 3/V). On one of the cells excited by IPNA, a depressant phase was revealed after the abolition of the excitatory response by phenoxybenzamine. On one cell, which did not respond to IPNA, a depressant response to IPNA appeared in the presence of phenoxybenzamine; this response disappeared after the application of the antagonist had been terminated.

We have also examined whether the  $\alpha$ -adrenoceptor blocking agents can discriminate between excitatory and depressant responses to adrenergic agonists on the same cell. In these experiments,

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\* An example of this observation is shown in Fig. 3/VII.



excitatory responses were evoked by phenylephrine, depressant responses were evoked by either IPNA or salbutamol, and phenoxybenzamine was used as the antagonist. Antagonism studies were successfully completed on 7 cells; on all these cells the excitatory response was antagonised, whereas the depressant response was not affected. A example of this observation is shown in Fig. 4/V.

b.  $\beta$ -Adrenoceptor blocking agents

The  $\beta$ -adrenoceptor blocking agent sotalol was effective in reversibly antagonising depressant responses to IPNA while responses to ACh were not affected (19 cells) (see Fig. 5/Va). On two cells depressed by IPNA, an excitation was revealed after the abolition of the depressant responses by sotalol. On one cell which did not respond to IPNA, an excitatory response appeared in the presence of sotalol; this response disappeared after the application of sotalol had been terminated. The  $\beta$ -receptor blocking agents, propranolol and sotalol could also reversibly antagonise excitatory responses to adrenoceptor agonists, without affecting responses to ACh: excitatory responses were antagonised by propranolol on 4 cells, and excitatory responses to IPNA were antagonised by sotalol on 2 cells (see Fig. 5/Vb).

We have also examined whether these antagonists can discriminate between depressant and excitatory responses to adrenergic agonists on the same cell. In these experiments, depressant responses were evoked by IPNA, excitatory responses were evoked by phenylephrine, and sotalol was used as the antagonist. Antagonism studies were successfully completed on 9 cells. On all these cells, the depressant



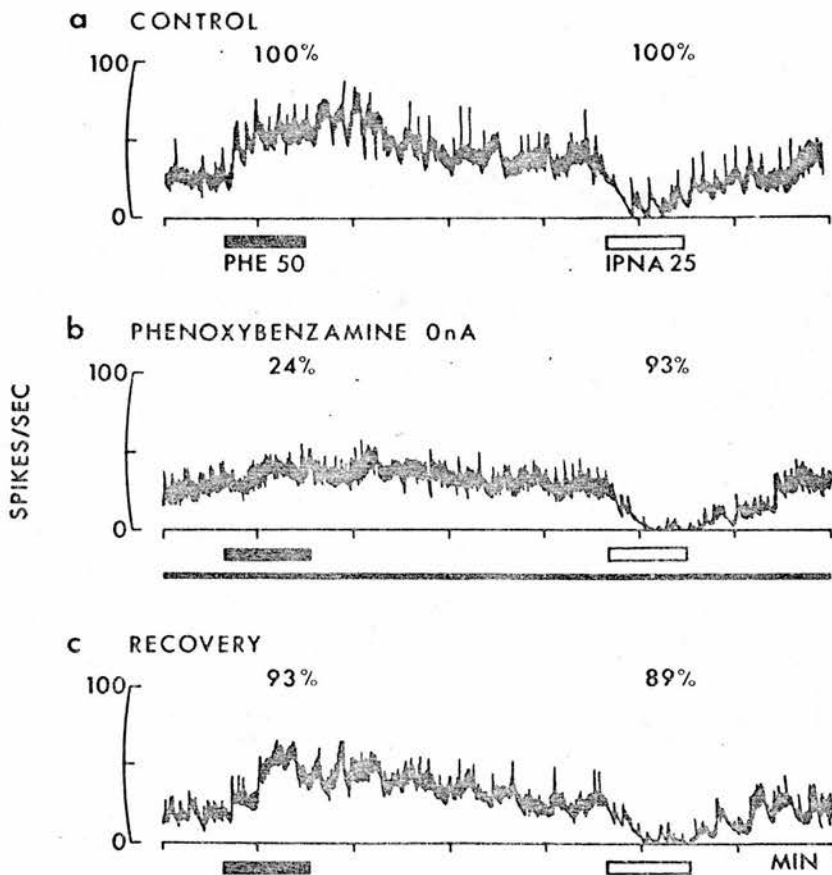
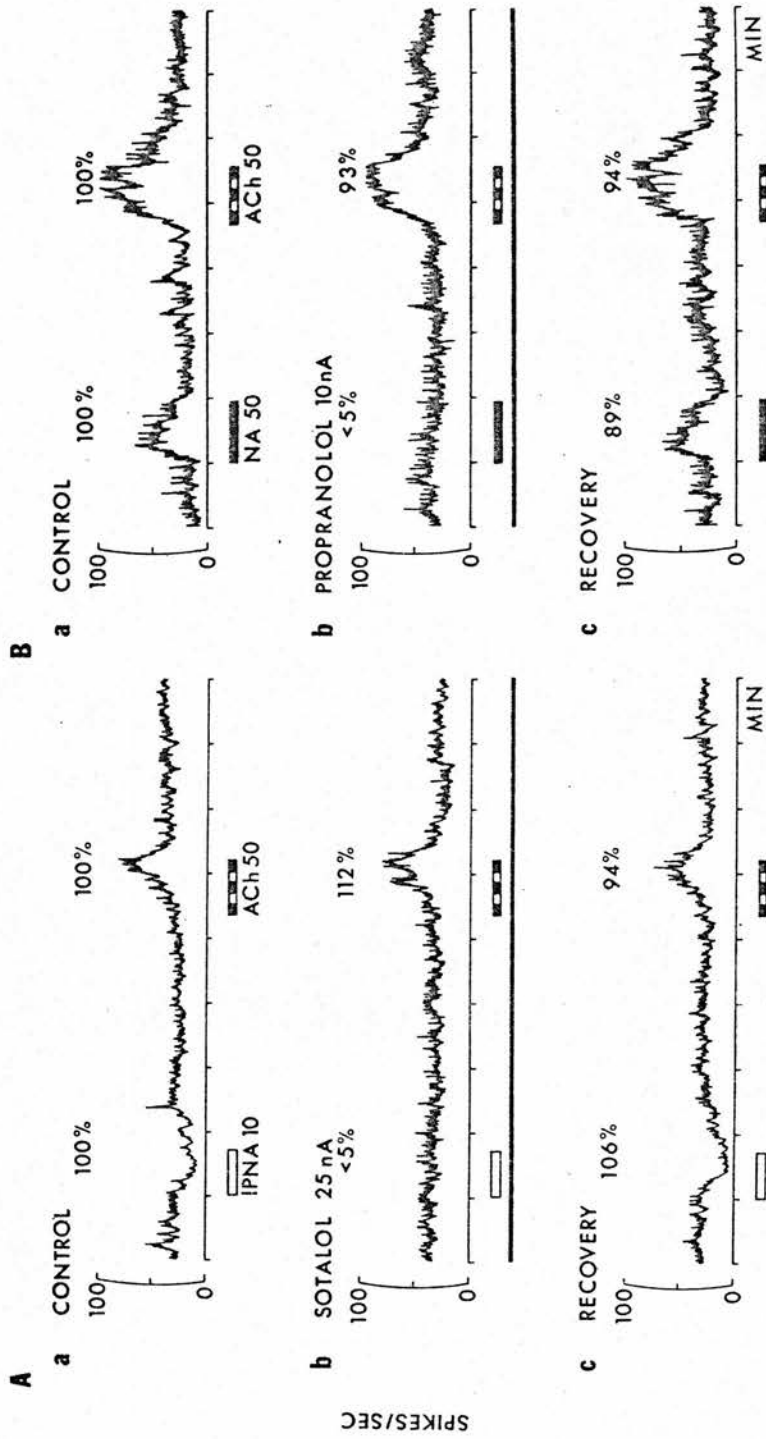


FIGURE 4/V. Effects of phenoxybenzamine on excitatory and depressant responses to adrenergic agonists.

Ratemeter recording of the firing rate of a single cortical neurone in the rat. Ordinates: firing rate (spikes/sec); abscissae: running time/min. Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents (nA). Figures above the traces indicate total spike numbers (%), taking the sizes of the control responses to each agonist as 100%.

- Control responses to phenylephrine (Phe) and isoprenaline (IPNA).
- Responses to the agonists during the continuous application of phenoxybenzamine. At the start of trace (b) phenoxybenzamine (0 nA) had been applied continuously for 15 min. The excitatory response to phenylephrine, but not the depressant response to IPNA was antagonised.
- Recovery of the response to phenylephrine 20 min after the application of phenoxybenzamine had been terminated.

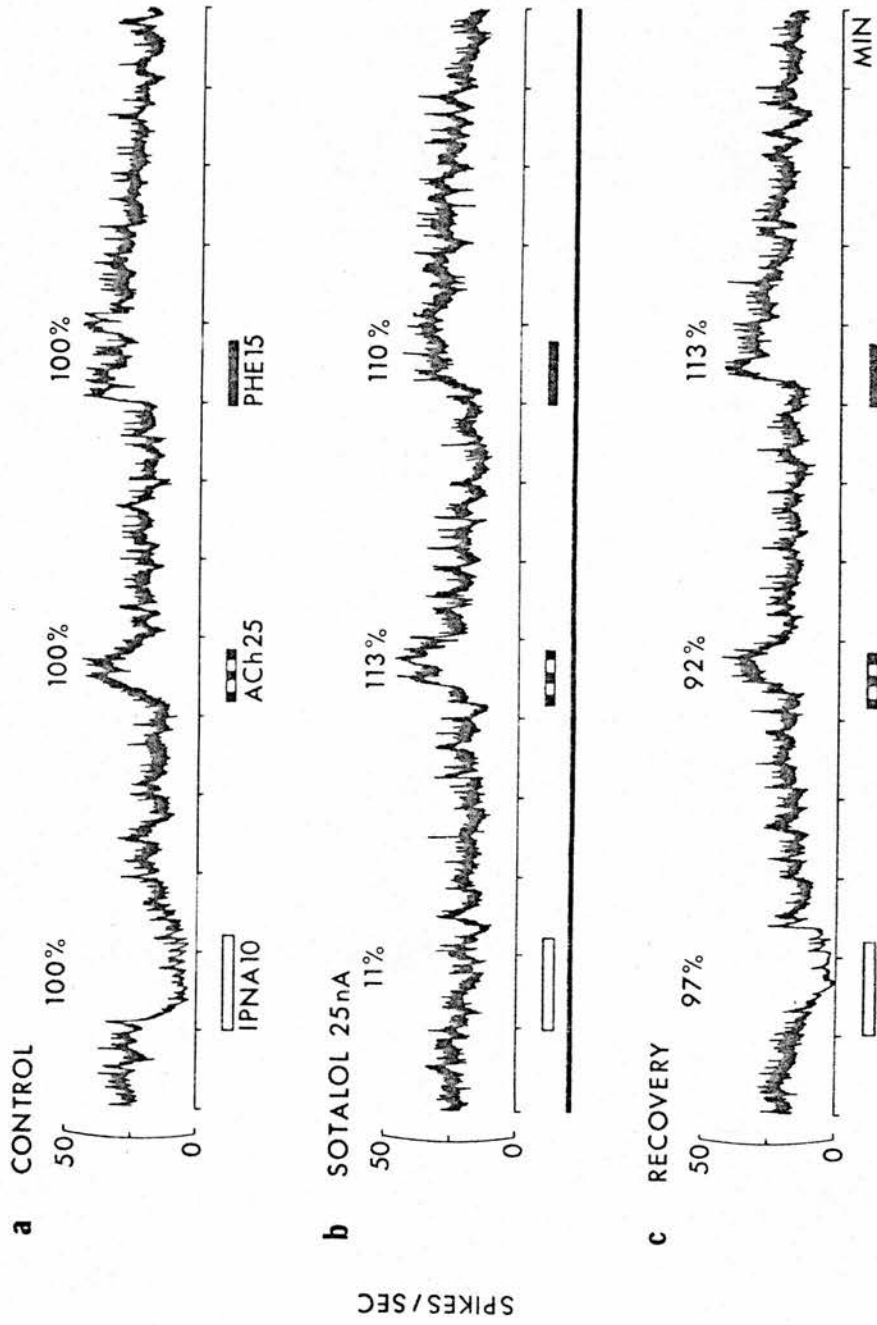


response was antagonised, whereas the excitatory response was not affected (see Fig. 6/V).

#### V.4. DISCUSSION

The action of adrenergic agonists (see Table 1/V) strongly suggests that the excitatory responses to these drugs are mediated by  $\alpha$ -, whereas the depressant responses are mediated by  $\beta$ -receptors. Phenylephrine (Furchgott, 1972; Besse & Furchgott, 1976) and methoxamine (Furchgott, 1970; Innes & Nickerson, 1975) are highly selective  $\alpha$ -adrenoceptor agonists, whereas salbutamol is a selective  $\beta$ -receptor stimulant with no affinity for  $\alpha$ -adrenoceptors (Brittain et al., 1970; Spedding & Weetman, 1972). On the other hand, IPNA and NA can act at both  $\alpha$ - and  $\beta$ -receptors (see Furchgott, 1972). It is well documented in the periphery that the dose-response curve to IPNA is a biphasic one: lower doses of the drug relax smooth muscle preparation due to the stimulation of  $\beta$ -receptors, whereas higher concentrations have a contractile effect due to the activation of excitatory  $\alpha$ -receptors (Spedding & Weetman, 1972; Trendelenburg, 1974). A very similar observation was made in our experiments: lower doses of IPNA depressed, whereas somewhat higher doses excited the same cortical neurone (see Fig. 2/V).

Methoxamine appeared to be less potent than phenylephrine in our experiments. This is in agreement with observations in the periphery where methoxamine has a considerably lower potency than phenylephrine (Furchgott, 1970; Schümann & Endoh, 1976). However, an apparent difference in potency in our experiments might also reflect physical factors, such as difference between the transport numbers and diffusion coefficients of the two drugs (see III.4.).



The results with the adrenergic antagonists support our suggestion, based on the action of the agonists, that excitatory responses are mediated by  $\alpha$ -, and depressant responses are mediated by  $\beta$ -receptors. In agreement with previous findings (Johnson et al., 1969a), we have found that the  $\alpha$ -adrenoceptor blocking agents could reversibly antagonise excitatory responses to adrenergic agonists, while excitatory responses to ACh were not affected. On the other hand, depressant responses to adrenergic agonists seemed to be resistant to  $\alpha$ -adrenoceptor blocking agents (see Fig. 4/V). This observation is in agreement with previous findings (Johnson et al., 1969a; Stone, 1973a), and probably reflects the failure of  $\alpha$ -adrenoceptor blocking agents to interact directly with  $\beta$ -receptors (Nickerson, 1967). Consequently, we were able to demonstrate that, on the same cell, the antagonism of excitatory responses to phenylephrine occurred when depressant responses were not affected (see Fig. 4/V). Occasionally, a depressant response could be revealed after the abolition of the excitatory response to IPNA, suggesting an action of IPNA at both types of receptor and also the lower affinity of the antagonist for inhibitory  $\beta$ -receptors. The selective blockade of excitatory  $\alpha$ -receptors may explain the observation that a depressant response to IPNA could be revealed by phenoxybenzamine on a cell which previously had not responded to IPNA; the failure of IPNA to evoke a response in the absence of phenoxybenzamine could have been due to a complete 'antagonistic agonism' resulting from the activation of the functionally opposite  $\alpha$ - and  $\beta$ -receptors (see Appendix).

The  $\beta$ -adrenoceptor blocking agents effectively abolished depressant responses without affecting excitatory responses to adrenergic agonists (see Fig. 6/V). These antagonists occasionally unmasked excitatory responses after the abolition of depressant responses to IPNA, or when IPNA alone was without any effect, indicating the greater sensitivity of the inhibitory  $\beta$ -receptors. The  $\beta$ -receptor blocking agents, however, were also capable of antagonising excitatory responses. This finding is in agreement with previous reports (Johnson et al., 1969a; Bevan et al., 1974a). This observation might seem to argue against our hypothesis that the excitatory responses are mediated by  $\alpha$ -receptors. There is, however, good experimental evidence in the periphery that the conventional  $\beta$ -receptor blocking agents can also block  $\alpha$ -receptors at somewhat higher concentrations than are required for selective  $\beta$ -receptor blockade (Patil et al., 1968; Gulati et al., 1969). On the basis of these observations, it might have been expected that higher ejecting currents would be needed to apply the  $\beta$ -adrenoceptor blocking agents in order to antagonise excitatory responses than to antagonise depressant responses. However, this was not apparent in the present results, and it is unlikely that such a difference could be detected using between-cell comparisons unless a considerably larger number of cells is studied. The differential sensitivities of inhibitory and excitatory receptors to  $\beta$ -adrenoceptor blocking agents may explain our previous observation that lower doses of sotalol often potentiate excitatory neuronal responses to NA, whereas higher doses have an antagonistic effect (Bevan et al., 1974a; 1974b).

The potentiation may reflect the selective blockade of masked inhibitory  $\beta$ -receptors, whereas the antagonism may reflect the blockade of both the  $\beta$ -receptors and the dominant excitatory  $\alpha$ -receptors (see also Appendix).

Similarly to the situation in many peripheral tissues (see Furchgott, 1972),  $\alpha$ - and  $\beta$ -receptors mediate opposite effects on cortical neurones, the  $\alpha$ -receptors being excitatory and the  $\beta$ -receptors being inhibitory. Moreover, most neurones studied provided evidence for the presence of both  $\alpha$ - and  $\beta$ -receptors (e.g. opposite effects of different agonists on the same cell, see Fig. 1/V; opposite effects of different doses of IPNA on the same cell, see Fig. 2/V; reversal of the response by an antagonist, see Fig. 3/Vb). Although the experimental evidence presented here does not indicate where these receptors are localised, the most parsimonious explanation seems to be that the two functionally opposing populations of  $\alpha$ - and  $\beta$ -receptors occur on the membrane of the same cell (see also IV.4.1.).

CHAPTER VI

COMPARISON OF NORADRENALINE AND DOPAMINE  
ON CORTICAL NEURONES

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### VI.1. INTRODUCTION

Single neurones in the cerebral cortex can respond both with excitation and depression to noradrenaline (NA) applied by microelectrophoresis (see Chapters IV and V). The experimental evidence presented in Chapter V suggests that these responses are mediated by  $\alpha$ - and  $\beta$ -adrenoceptors, the  $\alpha$ -adrenoceptors being excitatory, and the  $\beta$ -adrenoceptors being inhibitory.

Cortical neurones are also sensitive to dopamine (DA) applied by microelectrophoresis, both excitatory and depressant responses having been described (Bevan et al., 1975; Stone, 1976; Bunney & Aghajanian, 1976). It is well documented in the periphery that DA can stimulate both  $\alpha$ - and  $\beta$ -adrenoceptors, as well as specific DA receptors (Goldberg, 1975). It was of interest, therefore, to determine whether the actions of DA on cortical neurones are mediated by adrenoceptors, by DA receptors, or by both. In the experiments described in this Chapter, we have compared the actions of NA and DA on cortical neurones. We have also examined whether phenoxybenzamine (an  $\alpha$ -adrenoceptor blocking agent; Nickerson, 1967) and neuroleptics (proposed DA receptor blocking agents; Goldberg, 1975) can discriminate between excitatory responses to NA and DA.

## VI.2. METHODS

### VI.2.1. PHARMACOLOGICAL EXPERIMENTS

All the experiments described in this Chapter were conducted in rats anaesthetized with halothane. Our methods for the surgical preparation of the animals are described in IV.2.1. For the manufacture of six-barrelled micropipettes see II.2., for the methods of extracellular recording and microelectrophoretic drug application see III.2.3. and III.2.4.

Six-barrelled micropipettes of tip diameter 3-5  $\mu\text{m}$  were used. Two barrels of each micropipette contained 4 M NaCl, one for recording action potentials, the other for use in current balancing. The remaining barrels contained drug solutions. The following drug solutions were used: (-)-noradrenaline bitartrate (0.05 M, pH 3.0-3.5); dopamine hydrochloride (0.05 M, pH 4.0-4.5); acetylcholine chloride (0.05 M, pH 3.5-4.0); phenoxybenzamine hydrochloride (0.01 M, pH 3.0); haloperidol (0.01 M, dissolved in 0.01 M tartaric acid, pH 4.0);  $\alpha$ -flupenthixol dihydrochloride (0.01 M, pH 3.0);  $\beta$ -flupenthixol dihydrochloride (0.01 M, pH 3.0).

Spontaneously active neurones were studied in two areas of the cerebral cortex: a) prefrontal region (stereotaxic co-ordinates, according to König & Klippel (1963): A 9.8-11.4, L 0.5-2.4; b) parietal region: A 4.8-6.5, L 0.9-2.4). The area of recording was prepared as described previously (Bradshaw & Szabadi, 1972). The dura was either incised with a hypodermic

needle, or was penetrated directly with the micropipette. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered the agonists were applied in a regular cycle. Between successive applications of agonists retaining currents of -10 nA were passed. Retaining currents of -25 nA were used for the antagonists. Intervals between successive applications of the same agonist were kept constant in order to standardise the effects of the retaining current upon drug release during the ejection period (see II.3.3. and III.3.2.). The sizes of the neuronal responses to the agonists were expressed as the total number of action potentials produced in response to each drug application ('total spike number', see III.2.5.).

The effects of the antagonists were evaluated in the following way. When stable responses to the agonists had been obtained, the antagonist was applied continuously, either by removal of the retaining current (0 nA) thus allowing the drug to diffuse out from the micropipette, or by the passage of a weak ejecting current (5 to 10 nA), and the time-course of the ejecting current was increased until antagonism was observed. Then the application of the antagonist was terminated and the time-course of recovery was followed.

VI.2.2. MEASUREMENT OF THE RELEASE OF NORADRENALINE AND  
DOPAMINE FROM MICROPIPETTES IN VITRO

Six-barrelled micropipettes were used in these experiments. Three barrels of each pipette were filled with 0.05 M [carbinol- $^{14}\text{C}$ ] noradrenaline bitartrate; the remaining three contained [ethylamine-2- $^{14}\text{C}$ ] dopamine hydrochloride (Radiochemical Centre, Amersham). The specific activities of both solutions were 1.0 mCi/mmole.

Our methods for the collection of samples have been described in II.2. Ten minute sample collection times were used. Initially the rate of spontaneous release of radioactive material was measured in the absence of any electrophoretic current (4 samples). Then the rate of release of radioactive material was measured in the presence of ejecting currents of +25 nA (4 samples) and +50 nA (4 samples) passed through each of the three barrels containing one of the catecholamines. Then, after a further 4 samples of spontaneously released radioactivity had been collected, the process was repeated for the three barrels containing the other catecholamine. The mean disintegrations per minute (dpm) obtained from the 8 samples of spontaneously released material were subtracted from the dpm obtained from each sample followed in the presence of an ejecting current; the remaining dpm were used for calculating the rate of electrophoretic release. The transport number ( $n$ )

of each catecholamine was calculated from the following formula:

$$n = R_e zF/3i$$

where  $R_e$  is the rate of electrophoretic release (moles/sec),  $z$  is the valency ( $z = 1$  for both NA and DA),  $F$  is Faraday's constant, and  $i$  is the intensity of the ejecting current (amps) passed through each of the three barrels containing the catecholamine in question.

### VI.3. RESULTS

#### VI.3.1. COMPARISON OF THE AGONISTIC EFFECTS OF NORADRENALINE AND DOPAMINE

##### a. Direction of responses to noradrenaline and dopamine

The directions of the responses (excitation or depression) evoked by NA and DA were compared on 136 neurones (prefrontal region 63 cells; parietal region 73 cells). Every one of these cells responded in the same direction to the two catecholamines, 103 being excited by both drugs, and 33 being depressed by both drugs. This positive correlation between the effects of NA and DA is statistically significant ( $\chi^2$  test,  $p < 0.0001$ ).

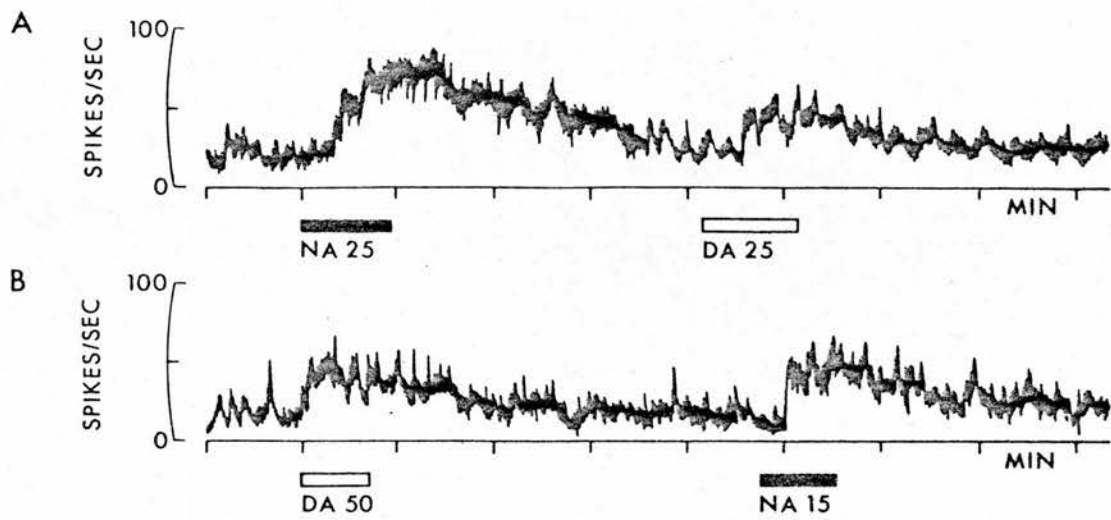
b. Apparent potencies of noradrenaline and dopamine

The relative potencies of NA and DA were assessed either by comparing the sizes of the responses evoked when the two drugs were applied with identical ejecting currents or in terms of the equipotent current ratio. The equipotent current ratio was defined as the ratio of the ejecting currents (current for DA/current for NA) needed in order to evoke responses of approximately equal magnitude (total spike numbers not differing by more than 20%) to the two catecholamines. In general, NA appeared to be more potent than DA; this was true both of excitatory and depressant responses. Of the 103 cells excited by both drugs, 70 (68%) showed evidence for a greater potency of NA (mean equipotent current ratio, 2.8), whereas on the remaining 33 (32%) no difference in potency could be observed. Of the 33 cells depressed by both drugs, 27 (82%) showed evidence for a greater potency of NA than DA (mean equipotent ratio, 3.2) whereas on the remaining 6 (18%) no difference in potency could be observed. Examples of these observations are shown in Fig. 1/VI. On none of the cells studied was DA observed to be more potent than NA.

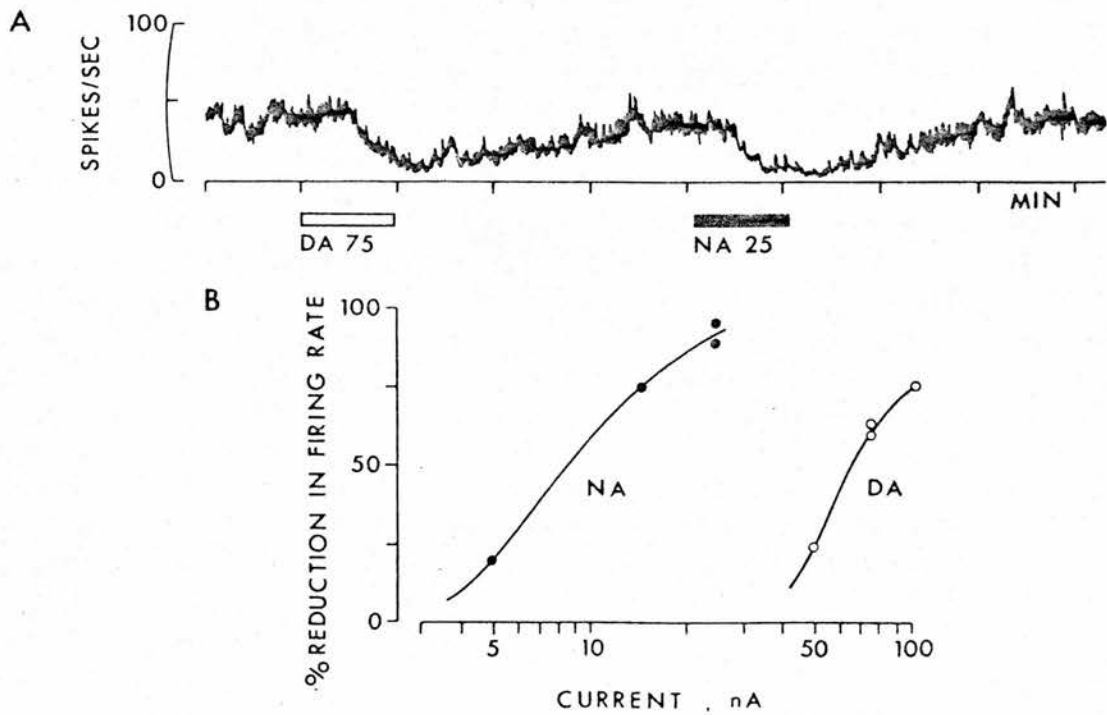
VI.3.2. EFFECTS OF ANTAGONISTS ON EXCITATORY RESPONSES TO NORADRENALINE AND DOPAMINE

In these experiments we compared the effects of the  $\alpha$ -adrenoceptor antagonist, phenoxybenzamine and the

## EXCITATORY RESPONSES



## DEPRESSANT RESPONSES



neuroleptic drugs, haloperidol,  $\alpha$ -flupenthixol and  $\beta$ -flupenthixol on excitatory responses to NA and DA. On every cell studied acetylcholine (ACh) was used as a control agonist.

The response to an agonist was regarded as antagonised if its size ('total spike number') was reduced by 50% or more in the presence of the antagonist (see Bevan et al., 1974a). An antagonist was regarded as having discriminated (or partially discriminated) between responses to two agonists if there was a discrepancy of 20% or more between the degree of antagonism observed on successive responses to the two agonists.

The results obtained from all the antagonist studies are summarised in Table 1/VI. Studies conducted in the prefrontal and in the parietal regions have been grouped together, since there was no detectable difference between the patterns of drug interaction observed in the two regions.

#### a. Phenoxybenzamine

The effects of phenoxybenzamine were examined on 16 cells. On all the cells tested phenoxybenzamine (10-10 nA) reversibly antagonised the response to NA without affecting the response to ACh. On 9 of the cells the response to DA was also antagonised; however, on the remaining 7 cells phenoxybenzamine discriminated between the responses to NA and DA, the response to DA being affected to a much smaller degree than



TABLE 1/VI. Summary of the effects of antagonists on excitatory responses to noradrenaline and dopamine

<u>antagonist</u>	<u>number of cells</u>			
	<u>response to NA</u> <u>affected more than</u> <u>response to DA</u>	<u>response to DA</u> <u>affected more than</u> <u>response to NA</u>	<u>responses to</u> <u>NA and DA</u> <u>equally affected</u>	<u>no</u> <u>effect*</u>
Phenoxylbenzamine	7	0	9	0
Haloperidol	0	8	3	1
$\alpha$ -flupenthixol	0	6	2	0
$\beta$ -flupenthixol	0	3	2	9

\* includes cells where only non-specific effects were seen (see text).

the response to NA (see Fig. 2/VI). On two cells, depressant responses to the catecholamines appeared when the excitatory responses were antagonised.

b. Haloperidol

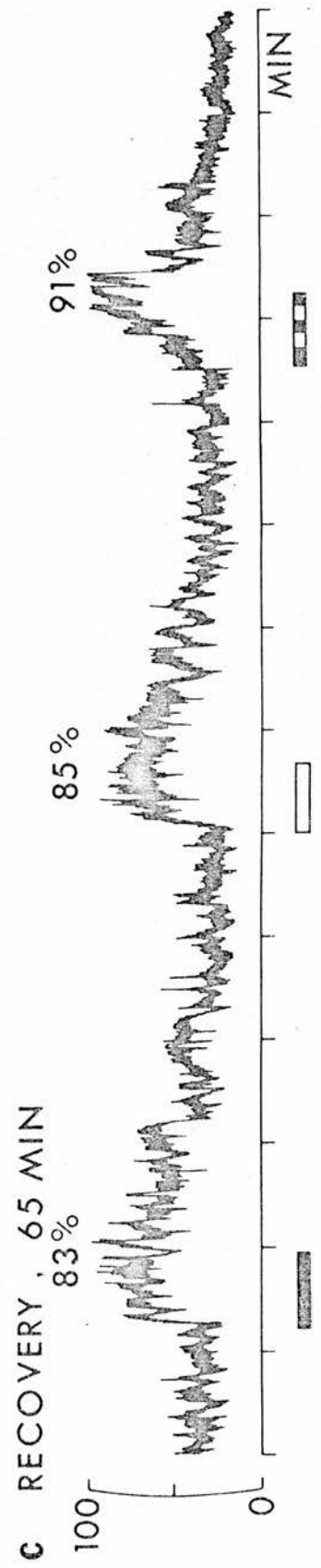
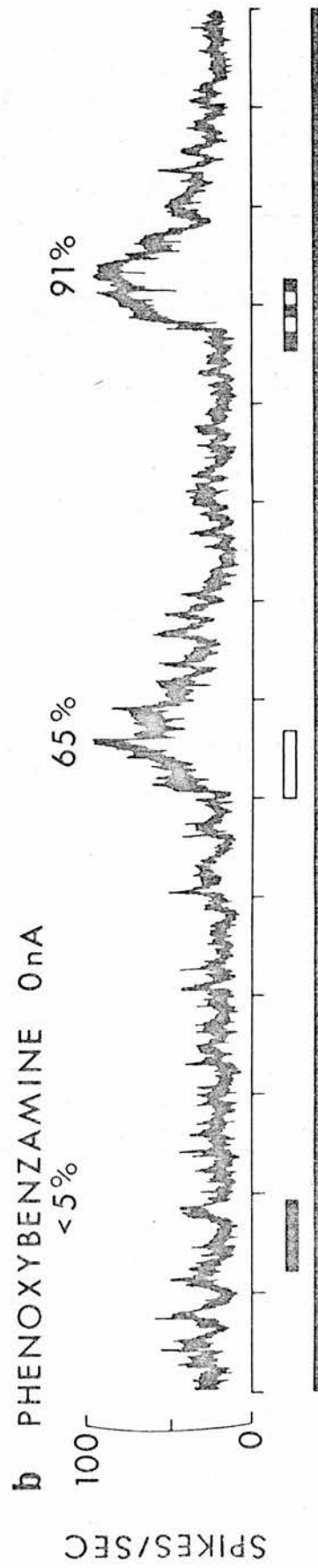
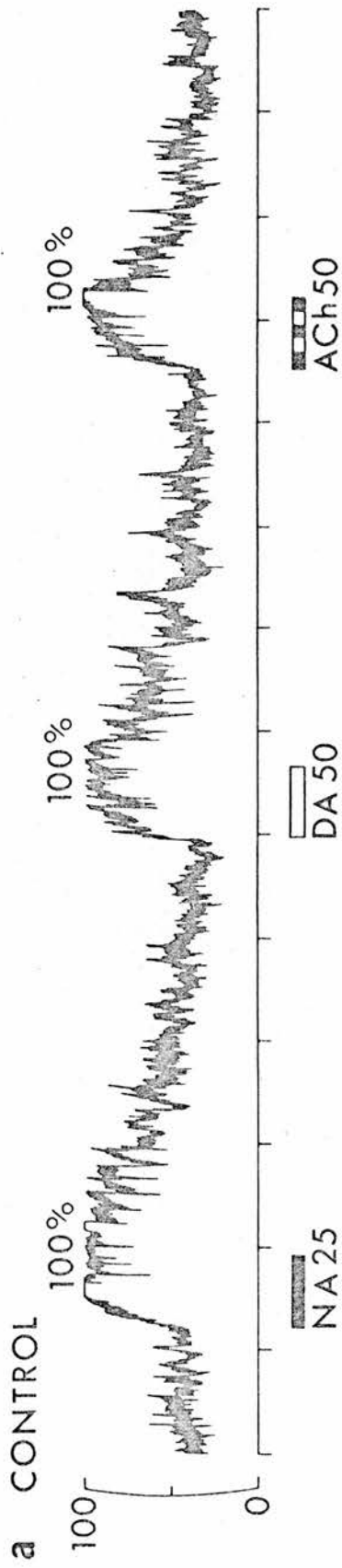
The effects of haloperidol were tested on 12 cells. On 11 cells haloperidol (5-20 nA) antagonised the response to DA when the response to ACh was not affected. On 3 of these cells the response to NA was antagonised equally to the response to DA; however, on the remaining 7 cells, haloperidol discriminated between the responses to the two catecholamines, the response to NA being affected to a lesser degree than the response to DA (see Fig. 3/VI).

c.  $\alpha$ -Flupenthixol

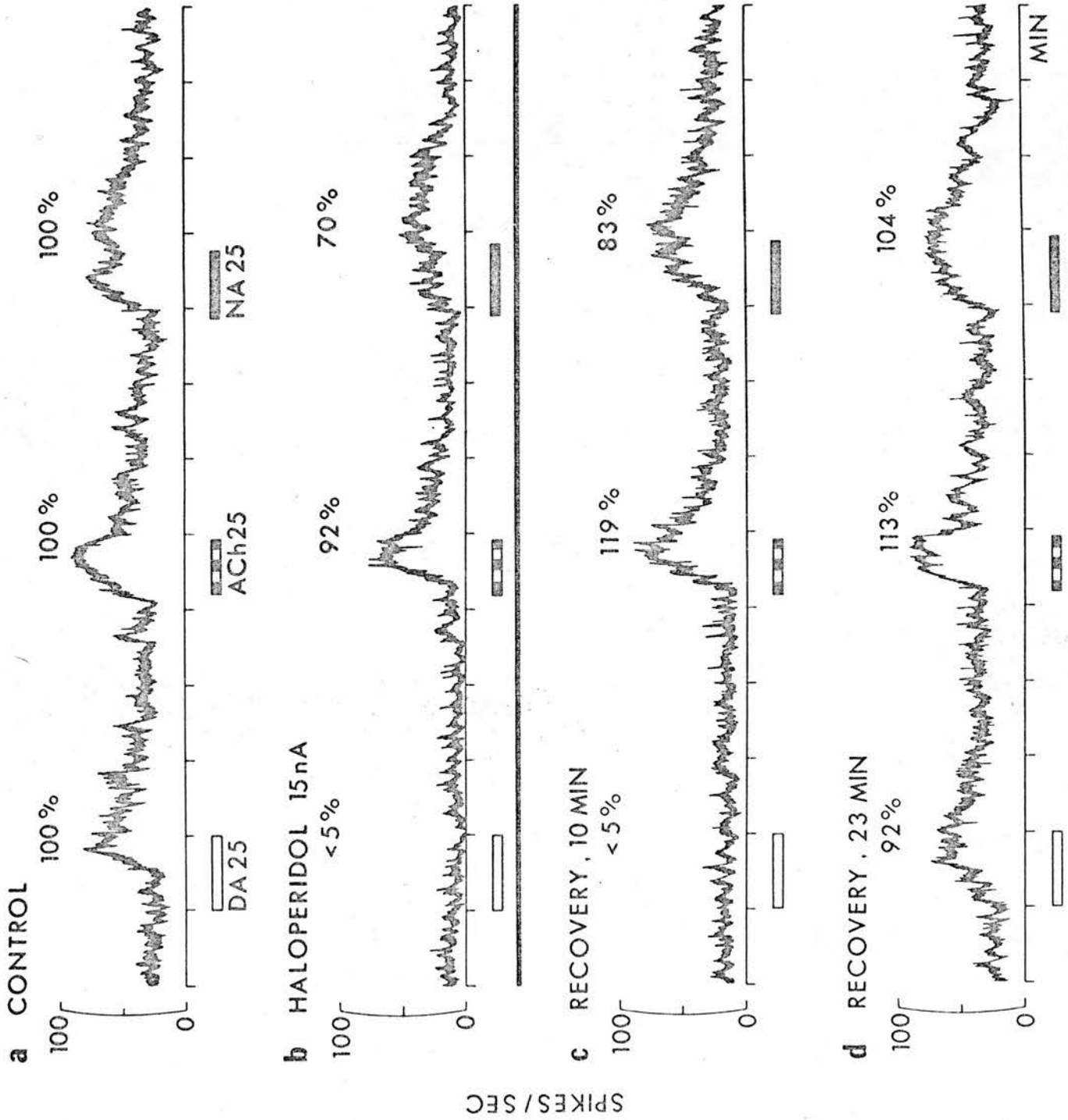
The effects of  $\alpha$ -flupenthixol were studied on 8 cells. In every case  $\alpha$ -flupenthixol (5-10 nA) antagonised the response to DA when the response to ACh was unaffected. On 2 cells the response to NA was affected equally to the response to DA; however, on the remaining 6 cells  $\alpha$ -flupenthixol partially discriminated between the responses to the two catecholamines, the response to NA being affected to a lesser degree than the response to DA (see Fig. 4/VI).

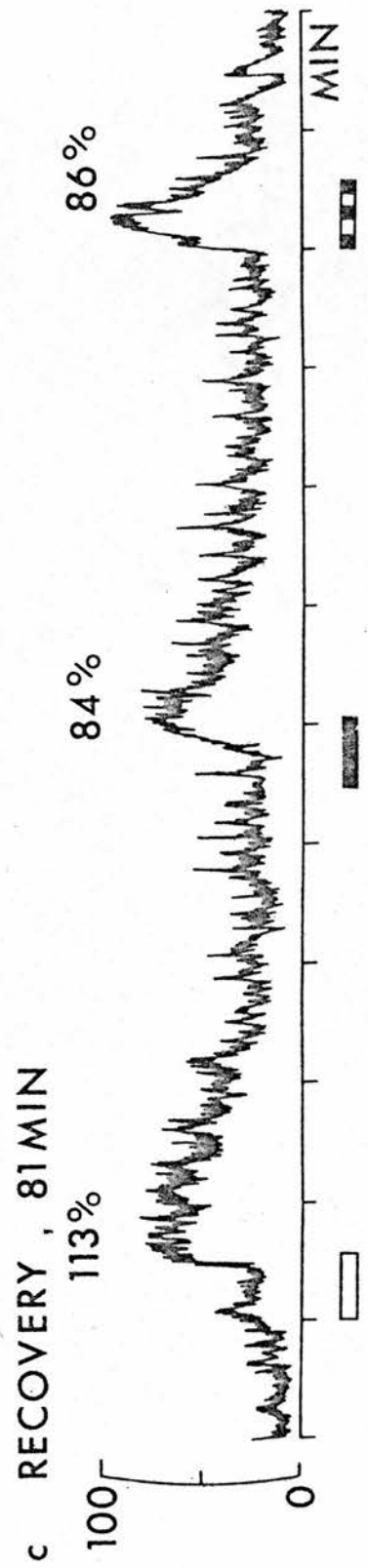
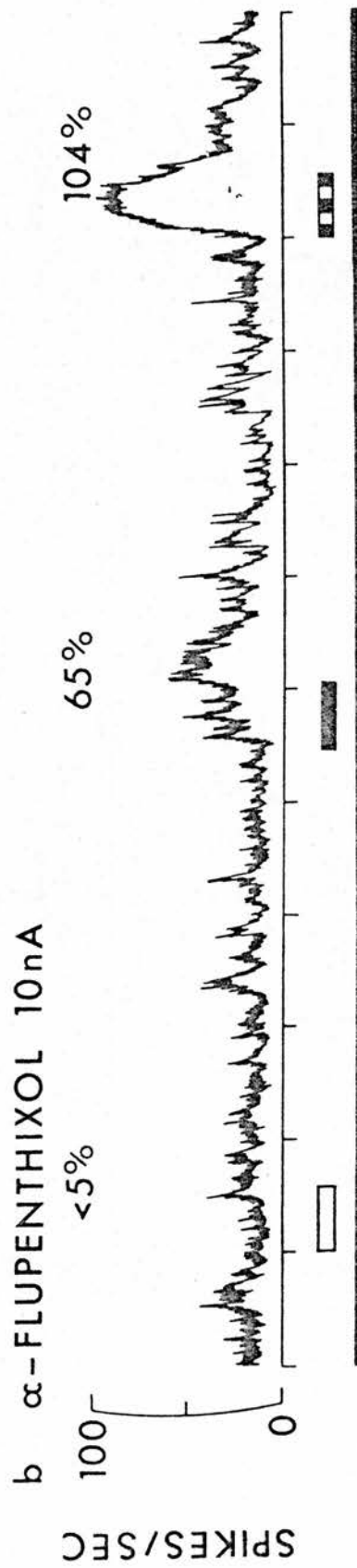
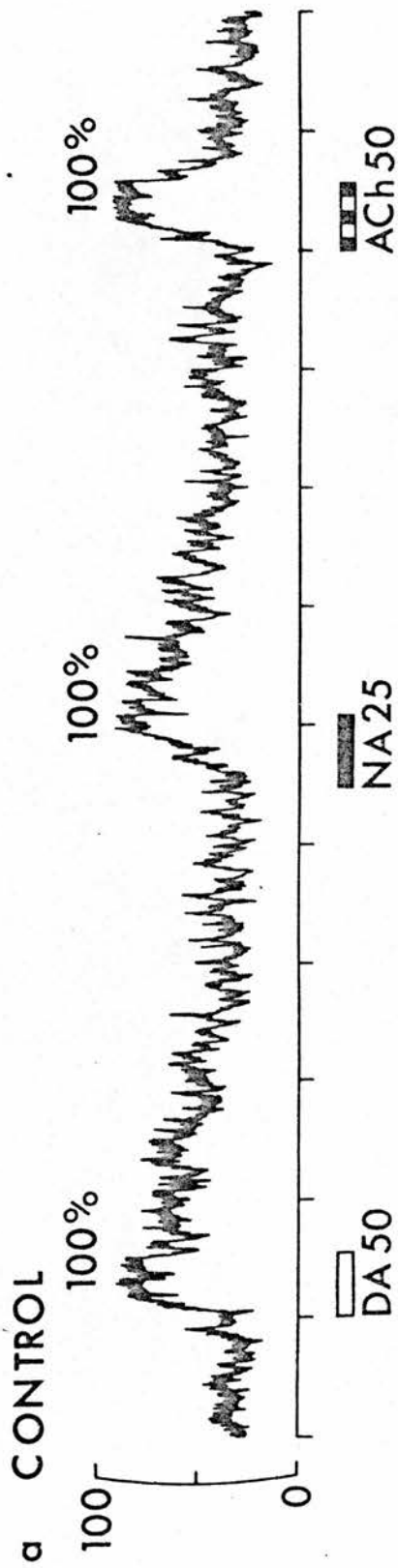
d.  $\beta$ -Flupenthixol

The effects of  $\beta$ -flupenthixol were studied on 14 cells. On 9 cells, flupenthixol (10-50 nA) was observed to have no



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specific effect, either failing to affect the responses to any of the agonists, or (especially when higher ejecting currents were used) reducing the responses to all three agonists and suppressing the spike amplitude. However, on the remaining 5 cells the responses to the catecholamines were antagonised when the response to ACh was unaffected: on 2 cells the responses to NA and DA were equally affected, whereas on the other 3 cells the response to DA was affected to a greater extent than was the response to NA.

#### VI.3.3. RELEASE OF NORADRENALINE AND DOPAMINE FROM MICROPIPETTES IN VITRO

The transport numbers of NA and DA were compared using 4 micropipettes; the results obtained are shown in Table 2/VI. In the case of each micropipette tested the transport number of DA was higher than that of NA; this difference was statistically significant in three of the four pipettes.

#### VI.4. DISCUSSION

In agreement with previous reports, the results presented in this Chapter show that catecholamines can evoke both excitatory and depressant responses on cortical neurones (Johnson et al., 1969; Bevan et al., 1974a, 1975). The

TABLE 2/VI. Transport numbers of noradrenaline and dopamine obtained from four six-barrelled micropipettes

<u>Pipette</u>	<u>tip diameter</u> ( $\mu\text{m}$ )	<u>transport number</u> ( $\text{mean} \pm \text{s.e.m. of 8 observations}$ )	<u>significance</u> <u>t test</u>
		NA	DA
1	3.5	0.288 ( $\pm$ 0.007)	0.300 ( $\pm$ 0.005) $0.1 > p > 0.05$
2	4.0	0.365 ( $\pm$ 0.013)	0.400 ( $\pm$ 0.011) $p < 0.02$
3	5.0	0.373 ( $\pm$ 0.009)	0.396 ( $\pm$ 0.007) $p < 0.02$
4	3.0	0.295 ( $\pm$ 0.008)	0.407 ( $\pm$ 0.016) $p < 0.001$

present results also indicate that NA and DA evoke qualitatively similar actions when tested on the same neurones, in that the direction of the responses evoked by the two drugs were invariably the same.

On most of the cells tested NA had a greater apparent potency than DA, and in no case did we observe DA to have a greater apparent potency than NA. In microelectrophoresis experiments a difference between the apparent potencies of two compounds may reflect physical rather than biological factors. For instance, a lower transport number of one compound will be reflected in a lower rate of release from the micropipette when both compounds are released with identical ejecting currents (see Curtis, 1964; see also Chapters II and III). However, the greater apparent potency of NA than DA seen in the present experiments probably reflects a genuine biological potency difference, since when the transport numbers of the two catecholamines were compared within the same micropipette the transport number of DA tended to be somewhat higher than that of NA.

On nearly half the cells tested, the  $\alpha$ -adrenoceptor blocking agent, phenoxybenzamine could discriminate between the excitatory responses to NA and DA; the responses to NA being more sensitive to the antagonistic effect of phenoxybenzamine than the responses to DA. The neuroleptics, haloperidol and  $\alpha$ -flupenthixol, drugs that are believed to block DA receptors



(Miller et al., 1974; Goldberg, 1975; Kelly & Miller, 1975), could also discriminate between excitatory responses to the two catecholamines; in this case, however, the responses to DA appeared to be more sensitive to the antagonists than the responses to NA. The partial selectivity of phenoxybenzamine and of the neuroleptics suggests that the excitatory responses to the catecholamines may be mediated by two populations of receptors. One population may be more sensitive to NA than to DA, and may be more readily blocked by phenoxybenzamine than by the neuroleptics; this population probably consists of  $\alpha$ -adrenoceptors. The other population of receptors may be more sensitive to DA than to NA, and may be more readily blocked by neuroleptics than by phenoxybenzamine; this population may be tentatively defined as a separate population of excitatory DA receptors.

On a substantial proportion of the cells tested, however, the antagonists failed to discriminate between responses to the two catecholamines. One possible explanation for this lack of selectivity could be that only one population of receptors was present on these cells, and that these receptors were activated by both agonists. An alternative possibility is that, although both kinds of receptor occurred on these cells, the antagonist - in the dose range used - was not specific for one receptor type.

In our experiments  $\alpha$ -flupenthixol was a more effective and more selective antagonist of DA-induced excitation than was  $\beta$ -flupenthixol. A similar difference in potency between the two isomers of flupenthixol has been noted in other test systems, for example the DA-stimulated cyclic AMP production in striatal homogenates (Miller, et al., 1974) and the rotational behaviour induced by amphetamine and apomorphine in rats with unilateral lesions of the substantia nigra (Kelly & Miller, 1975). It is noteworthy, however, that in the present experiments  $\beta$ -flupenthixol was observed to have a specific antagonistic effect on excitatory responses to DA on some cells.

In Chapter V we provided evidence that both  $\alpha$ - and  $\beta$ -adrenoceptors can occur on cortical neurones, the  $\alpha$ -receptors mediating excitation, and the  $\beta$ -receptors mediating depression. In the present experiments we observed that neurones which were depressed by NA could also be depressed by DA; however we did not study the effects of antagonists upon these depressant responses to DA. It remains to be determined, therefore, whether a separate population of inhibitory DA receptors exists on cortical neurones, or whether the depressant effects of DA are mediated, at least in part, by  $\beta$ -adrenoceptors.

C H A P T E R   V I I

COMPARISON OF NORADRENALINE AND DOPA ON CORTICAL NEURONES

### VII.1. INTRODUCTION

L-3, 4-dihydroxyphenylalanine (DOPA) is widely used in the treatment of Parkinson's disease. DOPA is the metabolic precursor of dopamine (DA), and it is generally assumed that DOPA exerts its therapeutic effect via the synthesis and release of DA in the caudate nucleus (Hornykiewicz, 1974). However, DOPA is also a precursor of noradrenaline (NA), and it has been suggested that in structures receiving a NA innervation, exogenously administered DOPA may cause the release of NA from presynaptic terminals and thus mimic the actions of NA on postsynaptic cells (Andén, Carlsson & Häggendal, 1969). In the experiments reported here we compared the effects of microelectrophoretically applied DOPA and NA on single neurones in the cerebral cortex, since this structure is known to be innervated by NA-containing neurones (Fuxe, 1965).

### VII.2. METHODS

The experiments were conducted on adult cats of either sex (2-3.5 kg) and on male albino Wistar rats (250-300 g). The animals were anaesthetized with halothane (0.5-1.2%). Our methods for the surgical preparation of the animals, for the manufacture of six-barrelled glass micropipettes, for extracellular recording

and for microelectrophoretic drug application, have been described in previous Chapters (II.2.; III.2.; IV.2.).

Micropipettes having tip diameters of 3-5  $\mu$  were used. The following drug solutions were used: L-3, 4-dihydroxyphenylalanine methylester HCl (0.05 M, pH 5.0), (-)-noradrenaline bitartrate (0.05 M, pH 3.0-3.5), acetylcholine chloride (0.05 M, pH 3.5-4.0), phentolamine mesylate (0.01 M, pH 4.5-5.5), propranolol HCl (0.01 M, pH 4.5-5.5), atropine sulphate (0.01 M, pH 5.5-6.0).

Only spontaneously active neurones were studied in these experiments. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive drug applications retaining currents of -10 nA were passed. Intervals between successive applications of the same agonist were kept constant in order to standardise the effects of the retaining current upon drug release during the ejection periods (see II.3.3. and III.3.2.). The sizes of the neuronal responses to the agonists were expressed as the total number of action potentials produced in response to each drug application ('total spike number' see III.2.5.).

Our method for the evaluation of the effects of antagonists is described in V.2. and VI.2.

### VII.3. RESULTS

#### VII.3.1. AGONISTIC EFFECT OF DOPA

##### a. Responses of cortical neurones to DOPA

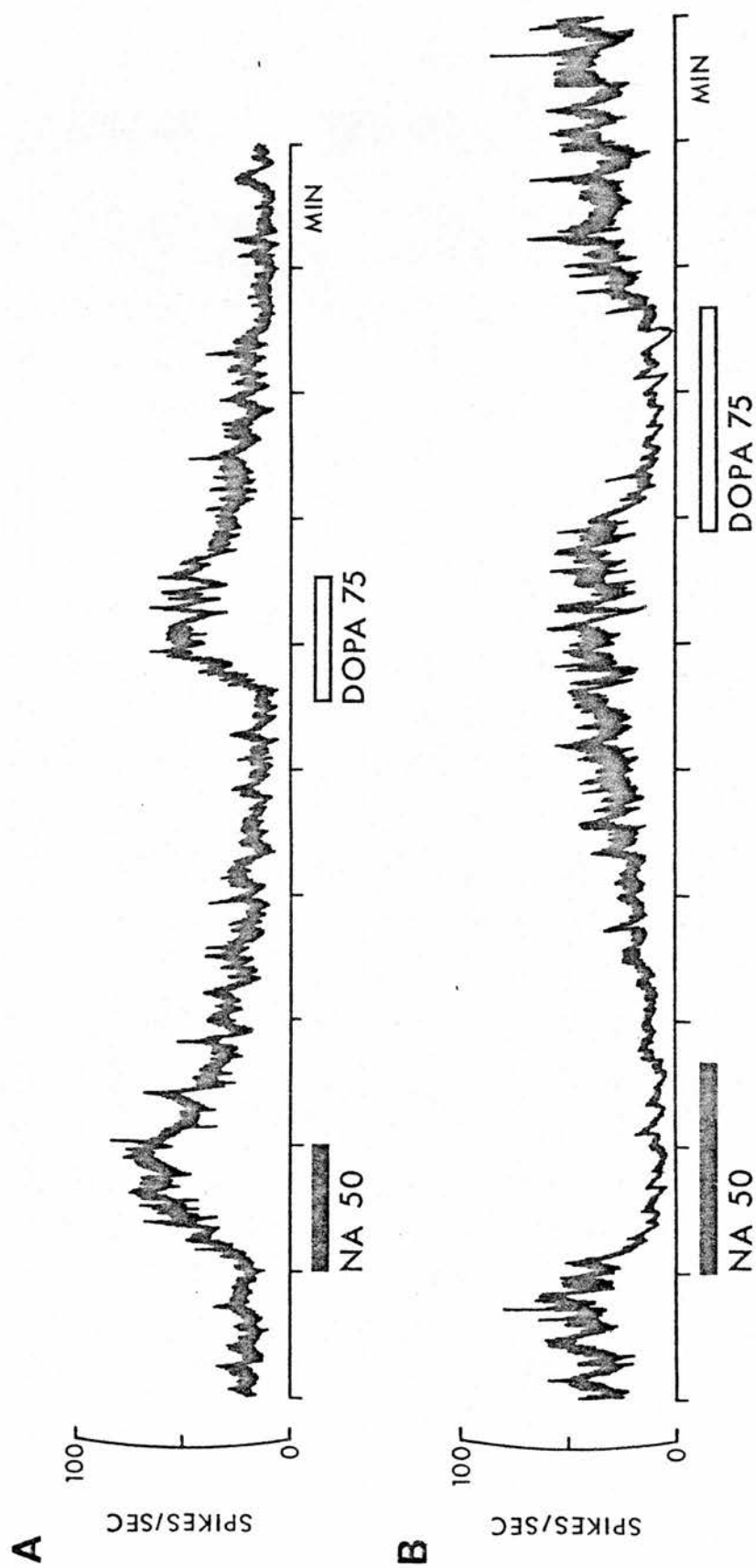
Both excitatory and depressant responses to DOPA were observed in these experiments. The effect of DOPA (25-150 nA, applied for 25-100 sec) was tested on 51 neurones in the rat; 44 were excited and 7 were depressed by DOPA. Eleven neurones were studied in the cat; of these 10 were excited and one depressed by DOPA. The predominance of excitatory responses in both species is statistically significant (binomial test,  $p < 0.01$  in both cases).

##### b. Direction of responses to DOPA and noradrenaline

Forty cells were studied which yielded consistent responses to both DOPA and NA (cat: 10 cells; rat: 30 cells). Every one of these cells responded in the same direction to the two drugs, cells excited by DOPA being also excited by NA and cells depressed by DOPA being also depressed by NA (Fig. 1/VII). This positive correlation between the effects of the two drugs is statistically significant ( $\chi^2$  test,  $p < 0.001$ ).

##### c. Comparison of apparent potencies of DOPA and noradrenaline

In 34 of the 40 cells on which the effects of DOPA and NA were compared, DOPA appeared to be less potent than NA



(binomial test,  $p < 0.01$ ). When DOPA and NA were applied to the same cell with identical ejecting pulses the size of the response (see Methods) evoked by NA was 1.8-3.4 times greater (inter-quartile range) than the response evoked by DOPA. In order to obtain approximately equivalent responses to the two drugs, the current needed to apply DOPA was 2.0-3.0 times greater (inter-quartile range) than that needed to apply NA. The lower potency of DOPA than NA was apparent in the case of both excitatory and depressant responses. Examples are shown in Fig. 2/VII.

d. Comparison of latencies to onset of responses to DOPA and noradrenaline

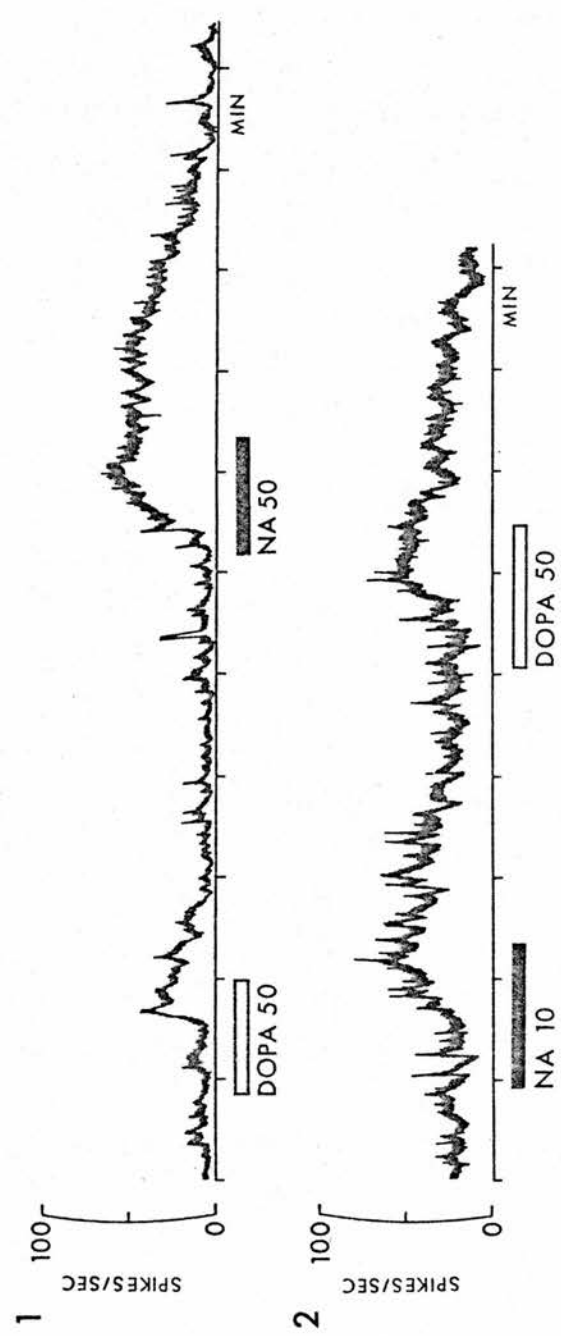
The latencies to onset<sup>of</sup> responses to DOPA and NA were compared in 12 cells to which the two drugs were applied with identical ejecting currents. The latencies of responses to both were 1.7-2.3 times greater (inter-quartile range), than those of responses to NA.

VII.3.2. EFFECTS OF ANTAGONISTS ON RESPONSES TO DOPA

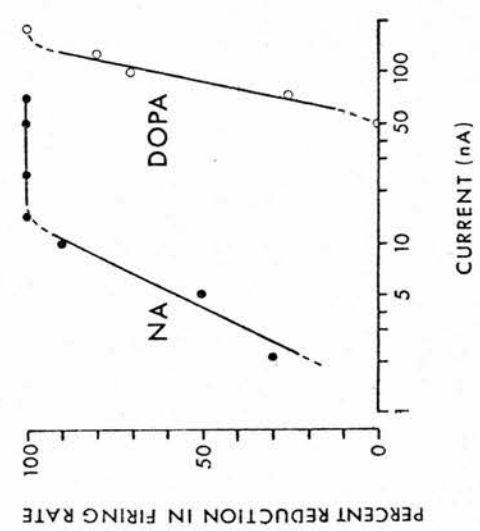
In these experiments the effects of the antagonists on responses to DOPA were compared with their effects on responses to NA and acetylcholine (ACh).



# A: EXCITATORY RESPONSES



# B: DEPRESSANT RESPONSES



#### a. Phentolamine

The effects of phentolamine were studied on 12 cells excited by DOPA (cat: 6 cells; rat: 6 cells). In all the cells tested, phentolamine (0-40 nA, applied for 10-60 min) reversibly antagonised excitatory responses to DOPA; excitatory responses to NA were also antagonised. These effects of phentolamine occurred at times when responses to ACh were not affected (see Fig. 3/VII). However, more prolonged applications of phentolamine were sometimes accompanied by loss of spike amplitude and reduced sensitivity to ACh.

In two cells in the rat, phentolamine failed to antagonise depressant responses to DOPA and NA.

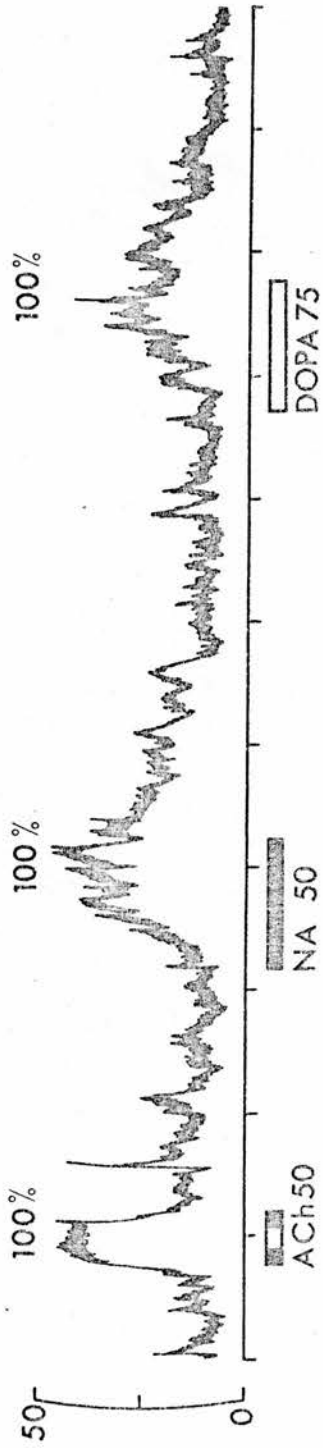
#### b. Propranolol

The effects of propranolol on excitatory responses to DOPA, NA and ACh were tested on 11 cells in the rat. In 8 of these cells propranolol (0-10 nA, applied for 10-40 minutes) reversibly antagonised the responses to DOPA and NA without affecting responses to ACh (see Fig. 4/VII). In the remaining 3 cells, propranolol produced a reduction in spike amplitude which precluded successful drug interaction studies.

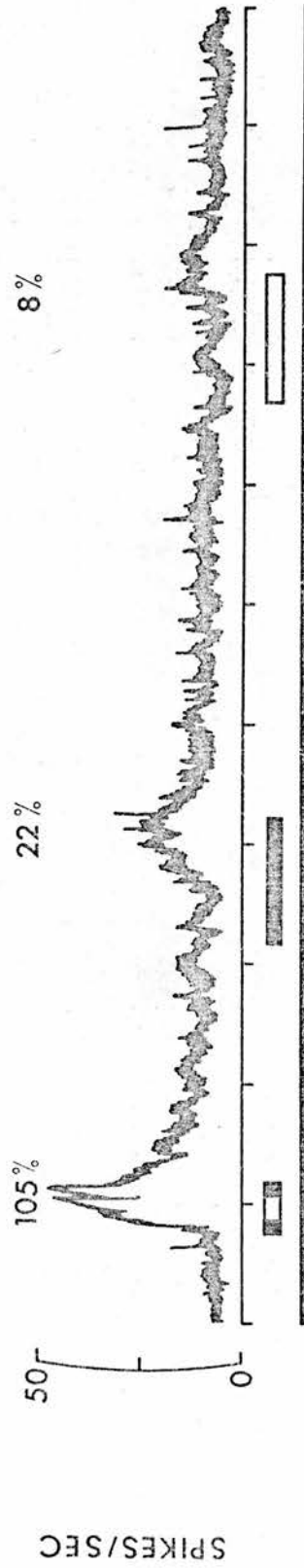
#### c. Atropine

The effects of atropine on excitatory responses to DOPA and ACh were tested on 6 cells in the rat. In all the cells

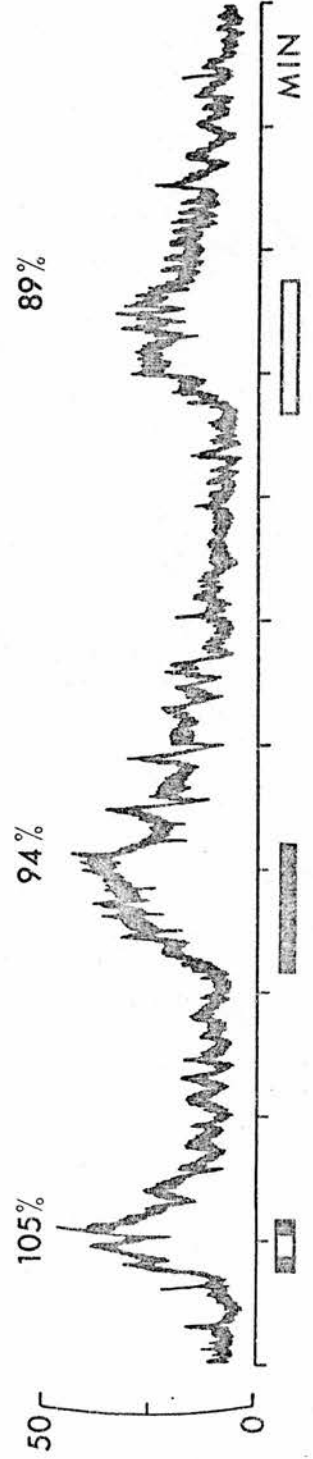
a CONTROL



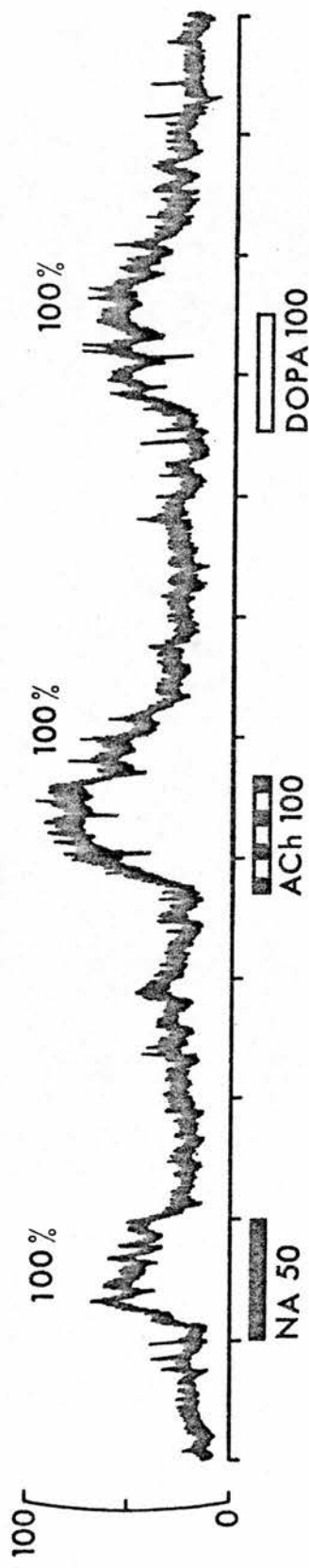
b PHENTOLAMINE 0 nA



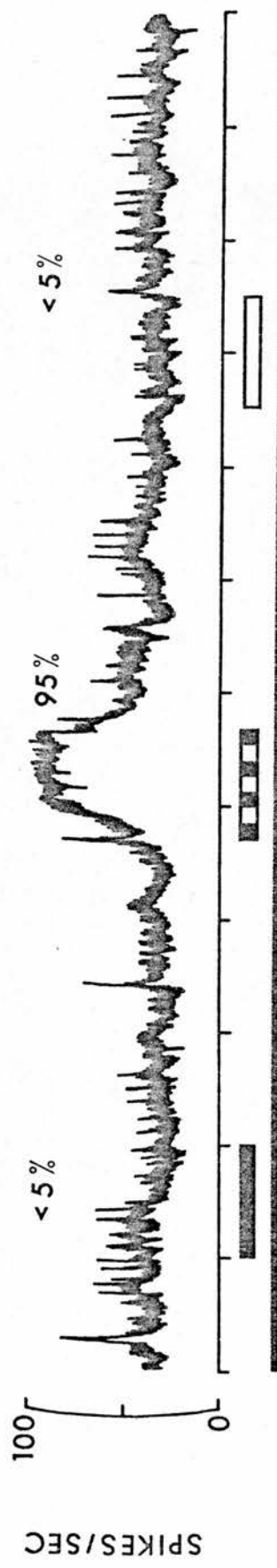
c RECOVERY



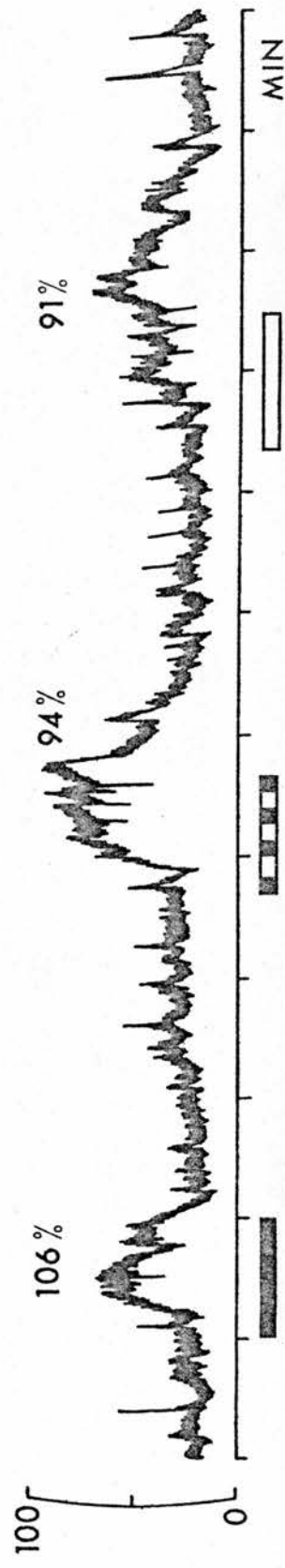
a CONTROL



b PROPANOLOL 10nA



c RECOVERY



tested, atropine (0-10 nA, applied for 10-30 min) reversibly antagonised the responses to ACh with little effect on responses to DOPA. An example of a cell on which the effects of both atropine and propranolol were studied is shown in Fig. 5/VII. On one cell atropine reversibly abolished the excitatory effects of ACh without affecting depressant responses to DOPA.

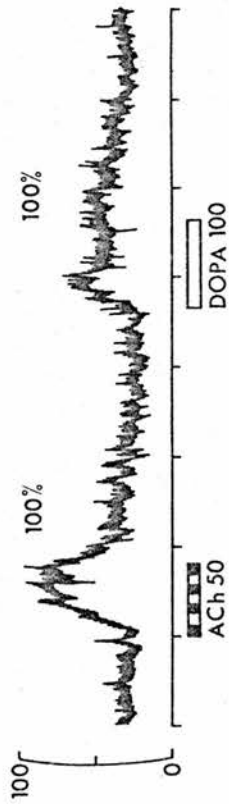
#### VII.4. DISCUSSION

The results reported here indicate that the effects of DOPA on single cortical neurones are very similar to those of NA. Firstly, it was found that cells invariably responded in the same direction to the two drugs. This contrasts with much lower correlations between the effects of NA and ACh, or NA and 5-HT on cortical neurones (Johnson et al., 1969a; Bradshaw et al., 1971; Bevan et al., 1974a). The present finding of a close correlation between the effects of DOPA and NA seems to be in contrast with the observations of Krnjević & Phillis (1963c) who reported that DOPA had a weak excitatory action on cortical neurones while NA was predominantly depressant. However, these authors did not report within-cell comparisons of the effects of the two drugs.

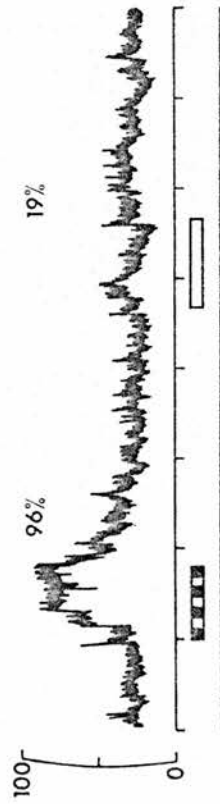
Secondly, the excitatory effects of both DOPA and NA could be abolished by phentolamine and propranolol when responses to ACh remained unaffected, while responses to DOPA were not affected by atropine when responses to ACh were completely abolished. The selectivity of these antagonists with respect

## A: PROPRANOLOL

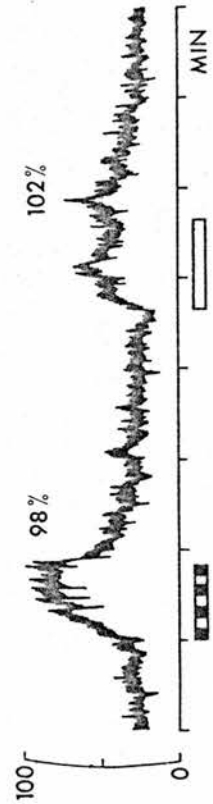
a CONTROL



b PROPRANOLOL 10nA

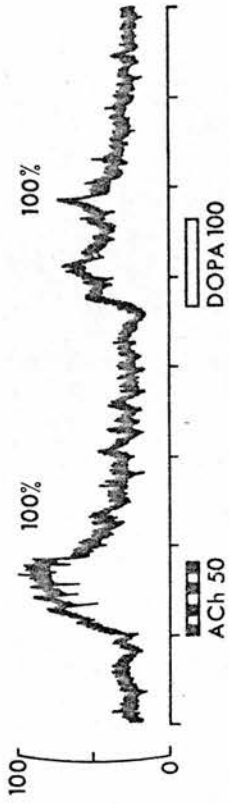


c RECOVERY

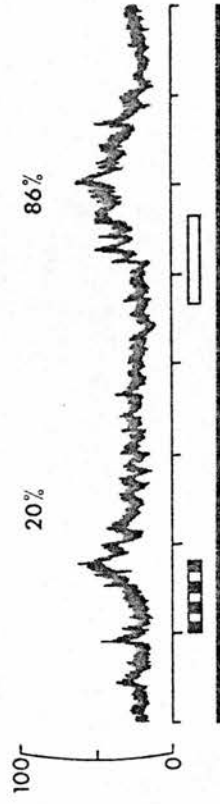


## B: ATROPINE

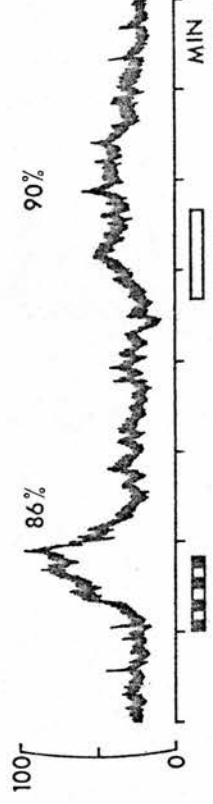
a CONTROL



b ATROPINE 10nA



c RECOVERY



to NA and ACh in our experiments is in agreement with the observations of Johnson et al., (1969a), and also with the observations presented in Chapter V. The evidence presented in Chapter V suggests that the excitatory responses to NA are mediated via  $\alpha$ -adrenoceptors. The selective antagonism of these responses by the  $\alpha$ -adrenoceptor blocking agent phentolamine supports this hypothesis. The antagonism of the excitatory responses to NA by propranolol, a  $\beta$ -receptor blocking agent, is probably due to the  $\alpha$ -adrenoceptor blocking property of this compound (for discussion see V.4.).

A possible explanation for the agonistic effects of DOPA seen in our experiments is the release of NA from noradrenergic nerve terminals. There is evidence that such a mechanism underlies the facilitatory effects of DOPA on spinal reflexes (Andén et al., 1969; Andén, Engel & Rubenson, 1972a) and the stimulant effects of DOPA on locomotor activity (Corrodi, Fuxe, Ljungdahl & Ögren, 1970). Since these actions of DOPA coincide with raised levels of NA in the CNS, and can be blocked by drugs which inhibit the synthesis of NA from DOPA, it is unlikely that DOPA exerts a direct action of its own in these test systems. However, in the present experiments, the possibility cannot be excluded that DOPA acted directly on post-synaptic NA receptors. (The observation that the latency

to onset of responses to DOPA was greater than that of responses to NA might be taken as evidence in favour of an indirect action of DOPA, however the dependence of response latency upon physical factors such as transport number, diffusion coefficient and the parameters of the ejecting and retaining currents (see Chapters II and III) makes such observations very difficult to interpret.)

An alternative possibility is that agonistic actions of DOPA were mediated by DA rather than NA. Recent evidence suggests that there are DA-containing nerve terminals in the cerebral cortex (Tierry, Hirsch, Tassin, Blanc & Glowinski, 1974; Fuxe, Hökfelt, Johansson, Jonsson, Lidbrink & Ljungdahl, 1974) and that DA can be synthesised in these terminals from systemically administered DOPA (Hökfelt, Ljungdahl, Fuxe & Johansson, 1974). Moreover, DOPA may be decarboxylated in 5 HT-containing nerve terminals, with the subsequent release of DA (Butcher, Engel & Fuxe, 1970). If, in the present experiments, DOPA acted either directly or indirectly on DA receptors, then the ant-agonistic effects of phentolamine and propranolol on responses to DOPA and NA would indicate that DA receptors are highly similar to NA receptors on cortical neurones (cf. Chapter VI).

Finally, some comment is needed about the apparently lower potency of DOPA than NA seen in our experiments. Such



a difference in potency might reflect physical factors, such as difference between the transport numbers and diffusion coefficients of the two drugs (see III.4.). However, the lower potency of DOPA might also be due to biological factors, such as the extra-neuronal accumulation of DOPA (Butcher et al. 1970; Andén et al., 1972b), the retention of newly synthesised catecholamines in presynaptic stores after DOPA administration (Butcher et al., 1970), or, if the primary action of DOPA is post-synaptic, difference between the affinities and/or intrinsic activities of the two drugs at NA receptors.

CHAPTER VIII

EFFECTS OF IMIPRAMINE AND DESIPRAMINE ON NEURONAL RESPONSES  
TO NORADRENALINE

#### VIII.1. INTRODUCTION

It is generally believed that the tricyclic antidepressant drugs exert their antidepressant effects by blocking the (re)uptake of noradrenaline (NA) into NA-containing nerve terminals, and thereby potentiating the pharmacological actions of NA on post-synaptic receptor sites (Schildkraut, 1965; Davis, 1970).

This hypothesis is based on observations made in the peripheral nervous system and in different peripheral pharmacological test systems. Tricyclic antidepressants (e.g. imipramine, desipramine) block the uptake of NA into sympathetically innervated tissues (Hertting et al., 1961; Iversen, 1965), and they are also able to potentiate the responses of adrenergically innervated tissues to exogenously applied NA (Sigg et al., 1963; Sturman, 1971; McCulloch & Story, 1972), and to sympathetic nerve stimulation (Sigg et al., 1963). It has been suggested that the potentiation of responses to NA is due to the blockade of uptake into nerve terminals (Hertting et al., 1961; Iversen, 1965; Schildkraut, 1965). As imipramine and desipramine block the uptake of NA into brain tissues as well (Ross & Renyi, 1967), it has been assumed that the tricyclic antidepressants should potentiate the pharmacological effects of monoamines in the brain (Schildkraut, 1965; Davis, 1970).

In the experiments described in this Chapter, we investigated how responses to NA can be modified by two tricyclic antidepressant drugs, imipramine and desipramine. For the sake of comparison, we have also examined the effects of desipramine on neuronal responses to glutamate.

The cerebral cortex is a relevant structure to conduct such an investigation. There is evidence that the neocortex receives innervation from NA-containing neurones (Fuxe, 1965; Descarries et al., 1977). Moreover, cerebral cortical neurones are sensitive to NA applied by microelectrophoresis: both excitatory and depressant responses have been described (see Chapters IV and V). It is possible, therefore, that these responses are mediated by subsynaptic receptors, and that presynaptic processes, such as uptake into NA-containing nerve terminals, may influence the responses observed.

## VIII.2. METHODS

All the experiments described in this Chapter were conducted on cats. Our methods for the preparation of the animals have been described in III.2.1. For the methods of manufacturing five-barrelled glass micropipettes see II.2. and III.2.2.; for the methods of recording action potentials and applying drugs by microelectrophoresis see III.2.3. and III.2.4.

The micropipettes were filled with the following drug solutions: noradrenaline bitartrate (0.2 M, pH 3.0-3.5), sodium glutamate (0.2 M, pH adjusted to 8.5 by the addition of 0.1 NaOH), imipramine hydrochloride (0.2 M, pH 4.4), desmethylimipramine hydrochloride (0.15 M, pH 4.5).

All the neurones studied were spontaneously active. All the drugs were applied by microelectrophoresis. Repeated responses to the monoamines (or glutamate) were compared before and after a brief application of the antidepressant. Our measure of the dose of the antidepressant was the electrophoretic charge passed (intensity of electrophoretic current x time of passage of current) (see VIII.4.). The sizes of excitatory responses to the agonists were expressed as the total number of spikes generated in response to each application of an agonist ('total spike number' ; see III.2.5.). In the case of depressant responses, the total number of spikes generated between the onset of the drug application and the recovery of the baseline firing rate was subtracted from the number of spikes emitted during an equivalent control period before the drug had been applied, and the figure obtained was used as a measure of the size of the depressant response. The intervals between drug applications were kept constant as far as possible. During these intervals a retaining current of 25 nA was passed.

### VIII.3. RESULTS

#### VIII.3.1. DIRECT EFFECTS OF IMIPRAMINE AND DESIPRAMINE ON NEURONAL FIRING

The direct effect of imipramine on the firing rate was studied on 144 cells; the effect of desipramine was investigated on 154 cells. The dose of antidepressant applied was 25-100 nA passed for 20-60 sec. The effects observed are summarized in Table 1/VIII. It is apparent that the vast majority of the cells (82.6 and 83.8%) were not directly affected by the antidepressants. The excitation or depression observed was always of a temporary nature, and the original baseline firing rate recovered within a minute after the application of the antidepressant had been terminated. On occasions a reduction in spike amplitude was observed; such cells were not used for drug-interaction studies.

On 29 cells a longer, continuous application of the antidepressants was also tested (5-50 nA passed for up to 20 min). This continuous application of the antidepressant resulted in a cumulative effect on firing rate: first the firing rate was gradually reduced, and later a decrease in spike amplitude developed. This progressively developing effect on spike amplitude could be observed even when very low ejecting currents were passed for a longer period. Therefore, a shorter application of the antidepressants was used for the drug-interaction studies described below.

TABLE 1/VIII. Effects of imipramine and desipramine on single cortical neurones

Effect (number of cells)				
	Excitation	Depression	Reduction in spike amplitude	No effect    Total
Imipramine	14 (9.8%)	8 (5.6%)	3 (2.0%)	119 (82.6%)    144 (100%)
Desipramine	9 (5.8%)	13 (8.4%)	3 (2.0%)	129 (83.8%)    154 (100%)

VIII.3.2. EFFECTS OF IMIPRAMINE AND DESIPRAMINE ON NEURONAL  
RESPONSES TO NORADRENALINE

a. Excitatory responses

Drug-interaction studies were successfully completed on 70 neurones responding with a clear increase in firing rate to NA. Cells were excluded if the variation in the size of response exceeded  $\pm 10\%$ . Imipramine was studied on 31 cells, desipramine on 39 cells. Both potentiation and antagonism of the response to NA could be observed after a brief application (25-100 nA for 20-60 sec) of either of the antidepressants.

Potentiation of the response was seen on 49 cells. A response was regarded as potentiated if there was more than 20% increase in the size of the average control response. The degree of potentiation was 51%-138% (inter-quartile range). The potentiated response had a characteristic time-course compared to the control response: the peak of the response was usually higher, and the recovery time longer. The latency of the potentiated response could be either shorter or longer than that of the control response. On a few cells only one response showed potentiation, on the majority of the cells, however, several responses were potentiated, and the control response recovered only after a longer time (up to 90 min).

Antagonism of the response was seen on 45 cells. Antagonism appeared as a reduction in the total spike number

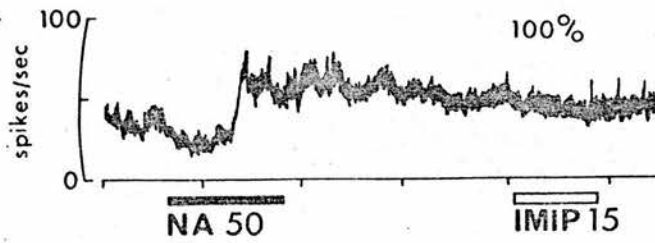


compared to the control. This reduction in size varied between 20-100%.

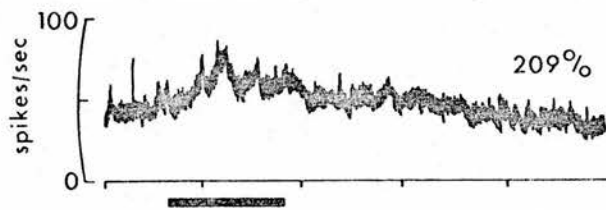
The occurrence of potentiation and antagonism followed a well-defined pattern. The following patterns could be observed:

1. 'Early' potentiation. In this case the first response after the antidepressant showed the greatest degree of potentiation. (The first application of NA after the antidepressant usually followed less than one and half minutes after the application of the antidepressant.) Subsequent responses became gradually smaller until recovery of the control could be seen. Recovery usually occurred 10-20 min after the antidepressant had been applied. Early potentiation was seen on 18 neurones.
2. 'Late' potentiation. In this case, potentiation developed gradually, achieving a maximum 10-30 min after the application of the antidepressant. Recovery occurred 30-60 min after the antidepressant had been applied. Late potentiation was seen on 18 cells. Examples of late potentiation are shown in Figs. 1/VIII, 3/VIII and 4/VIII.
3. Antagonism followed by potentiation. In this case, the first response after the antidepressant was reduced in size. This initial antagonism was later followed by potentiation, and finally by gradual recovery of the response. This pattern of drug-interaction was observed on 31 cells. Antagonism invariably preceded potentiation, the reverse was never seen. An example of this type of drug-interaction is shown in Fig. 2/VIII.

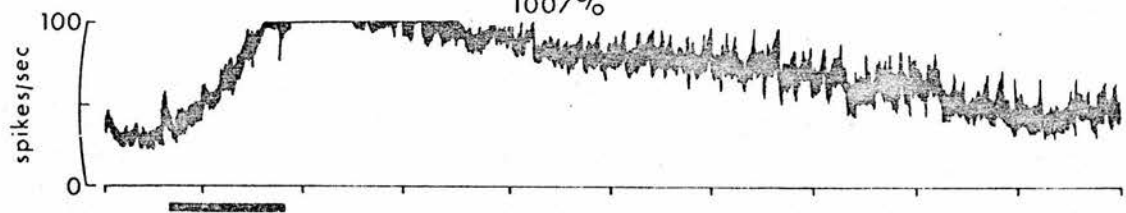
**a** CONTROL



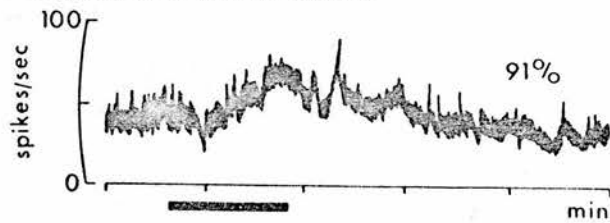
**b** 1/2 MIN AFTER IMIPRAMINE



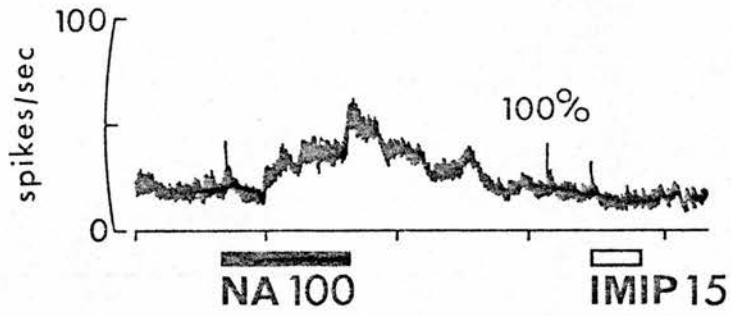
**c** 5 MIN AFTER IMIPRAMINE



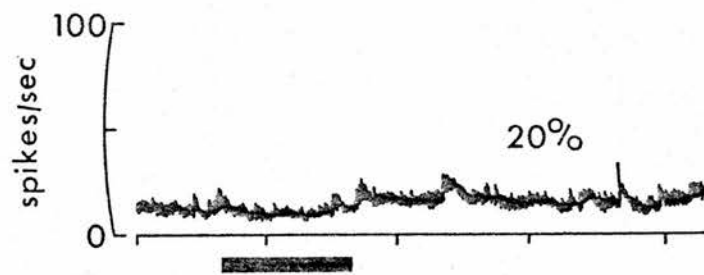
**d** 20 MIN AFTER IMIPRAMINE



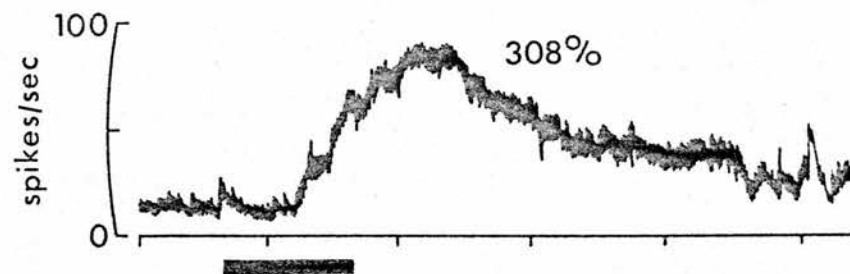
**a** CONTROL



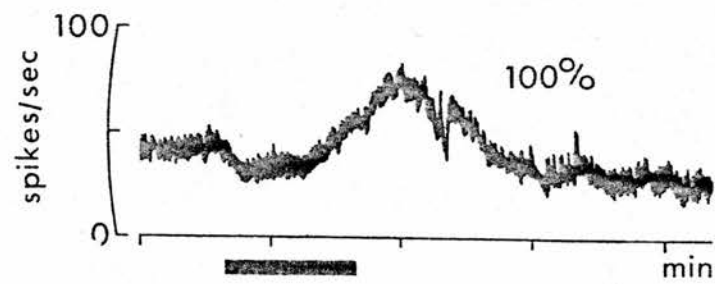
**b** 1 MIN AFTER IMIPRAMINE



**c** 10 MIN AFTER IMIPRAMINE



**d** 17 MIN AFTER IMIPRAMINE

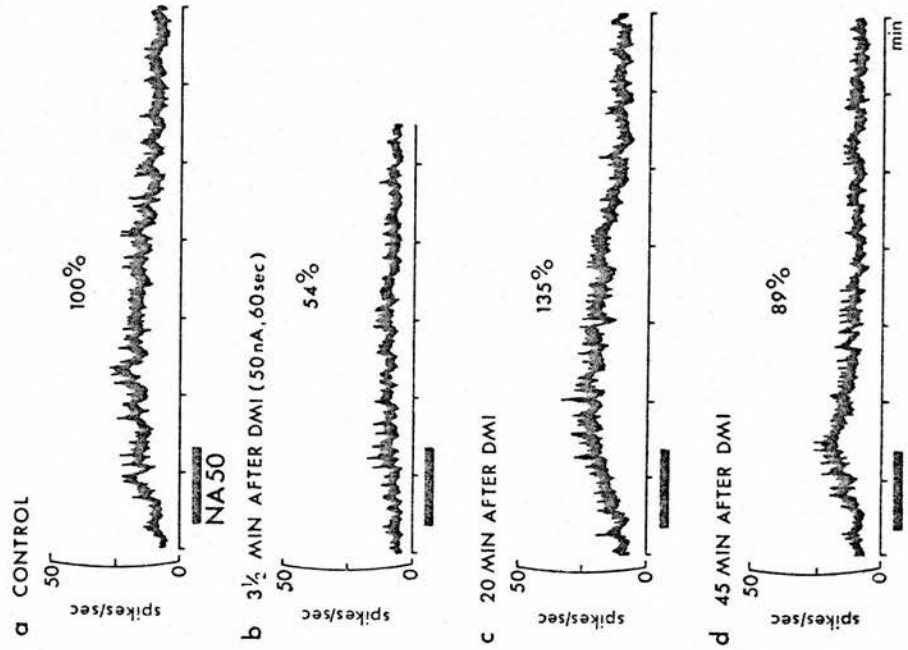


4. Antagonism only was seen on 14 cells. In this case an initial antagonism of the response was followed by recovery. On some cells a number of studies were conducted and more than one pattern of drug-interaction could be observed (see below).

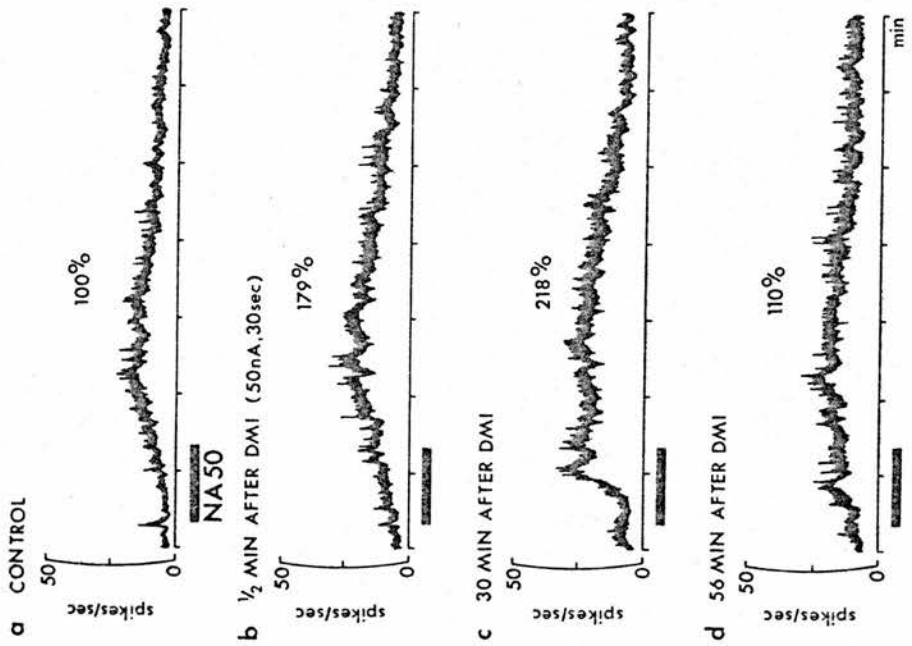
There was no qualitative difference between the effects of imipramine and desipramine: both antidepressants modified the response according to one of the four patterns.

In an attempt to identify whether the pattern of drug-interaction was related to the dose of antidepressant applied, we compared the effects of two or more doses of the same antidepressant on responses to noradrenaline on the same cells. The doses of antidepressant applied were within the range used in all of the drug-interaction studies. Increasing doses of imipramine were tested on 6 cells, and the effects of increasing doses of desipramine were studied on 12 cells. We have found that 1. a higher dose was required to cause late potentiation than early potentiation; 2. a higher dose was required to cause antagonism than potentiation only. An example of the effects of the two different doses of desipramine on the size of excitatory responses to noradrenaline is shown in Figs. 3/VIII and 4/VIII.

II



I



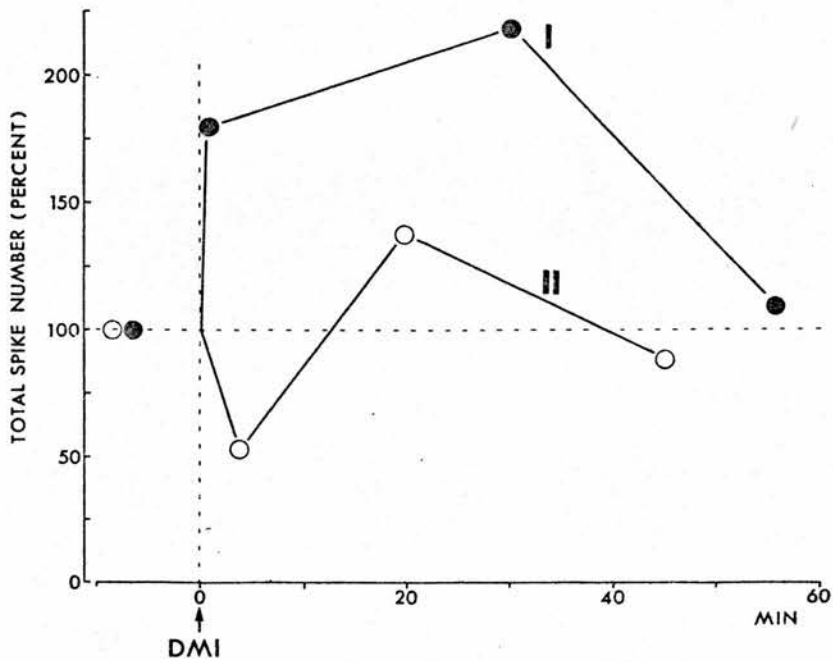


FIGURE 4/VIII. Effects of two 'doses' of desipramine on neuronal responses to noradrenaline.

Ordinate: total spike number (%; the size of the control responses is taken as 100%); abscissa: running time (min). Each dot represents one individual response; closed circles; responses from study I in Fig 3/VIII; open circles : responses from study II in Fig. 3/VIII.

The smaller dose of desipramine (50 nA for 30 sec) caused 'late potentiation', whereas the bigger dose of desipramine (50 nA for 60 sec) caused antagonism followed by potentiation.

#### b. Depressant responses

The effects of the two antidepressants were studied on 11 cells depressed by noradrenaline. Imipramine was studied on 6 cells, and desipramine was tested on 8 cells. Both potentiation and antagonism of the depressant responses after a brief application of the antidepressants could be observed (Fig. 5/VIII and 6/VIII). Potentiation was seen on 12 cells, antagonism was observed on 3 cells. The same patterns of drug-interaction were seen as with excitatory responses:

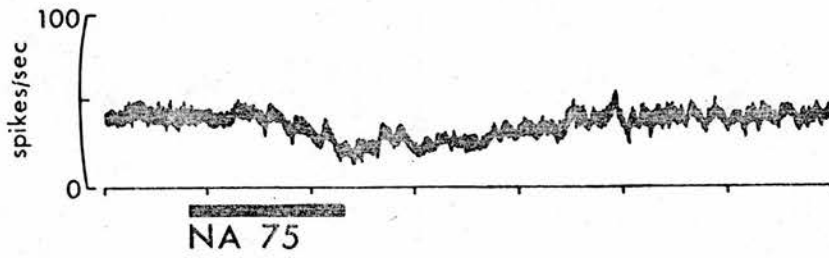
1. early potentiation (3 cells); 2. late potentiation (7 cells);
3. antagonism followed by potentiation (2 cells); 4. antagonism only (1 cell). There was no qualitative difference between the effects of imipramine and desipramine.

On 1 cell imipramine reversed the depressant response into an excitation (Fig. 7/VIII).

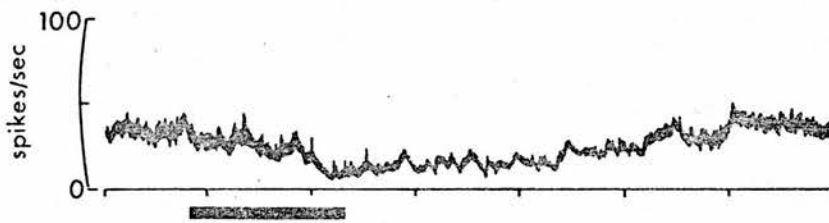
#### VIII.3.3. EFFECT OF DESIPRAMINE ON NEURONAL RESPONSES TO GLUTAMATE

The effect of desipramine on excitatory responses to glutamate was studied on 9 cells. On most of these cells several different doses of desipramine were tested. The dose of desipramine varied between 10-100 nA applied for 20-300 sec. The effects of glutamate on the firing rate were tested for 10-20 min following the application of the antidepressant. On none of the cells could any change be observed in the size of time-course of responses to glutamate during or after the application of desipramine.

a CONTROL



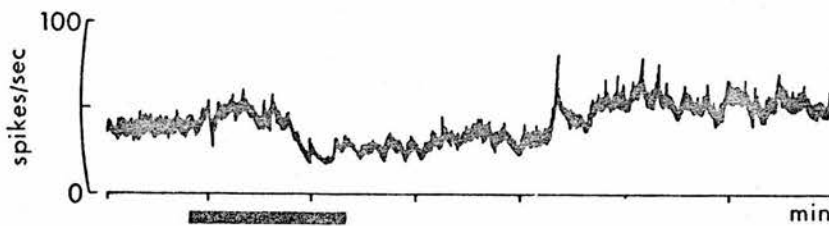
b 1½ MIN AFTER IMIPRAMINE (50nA, 35sec)



c 16 MIN AFTER IMIPRAMINE

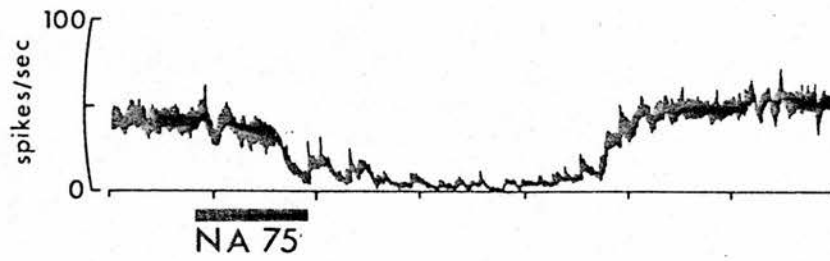


d 39 MIN AFTER IMIPRAMINE

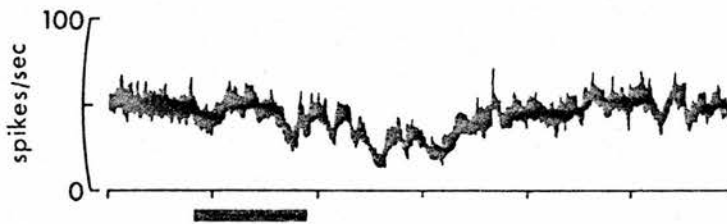




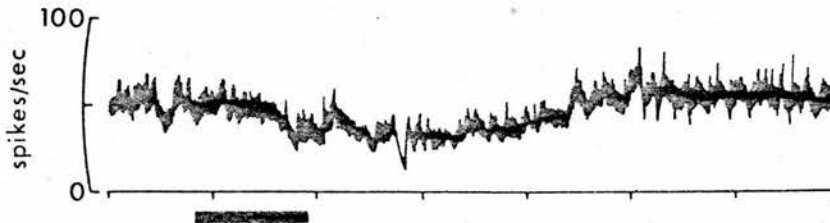
a CONTROL



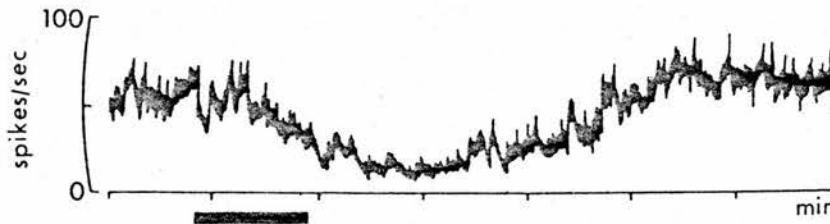
b  $\frac{1}{2}$  MIN AFTER IMIPRAMINE (50 nA, 35 sec)



c  $5\frac{1}{2}$  MIN AFTER IMIPRAMINE



d  $18\frac{1}{2}$  MIN AFTER IMIPRAMINE



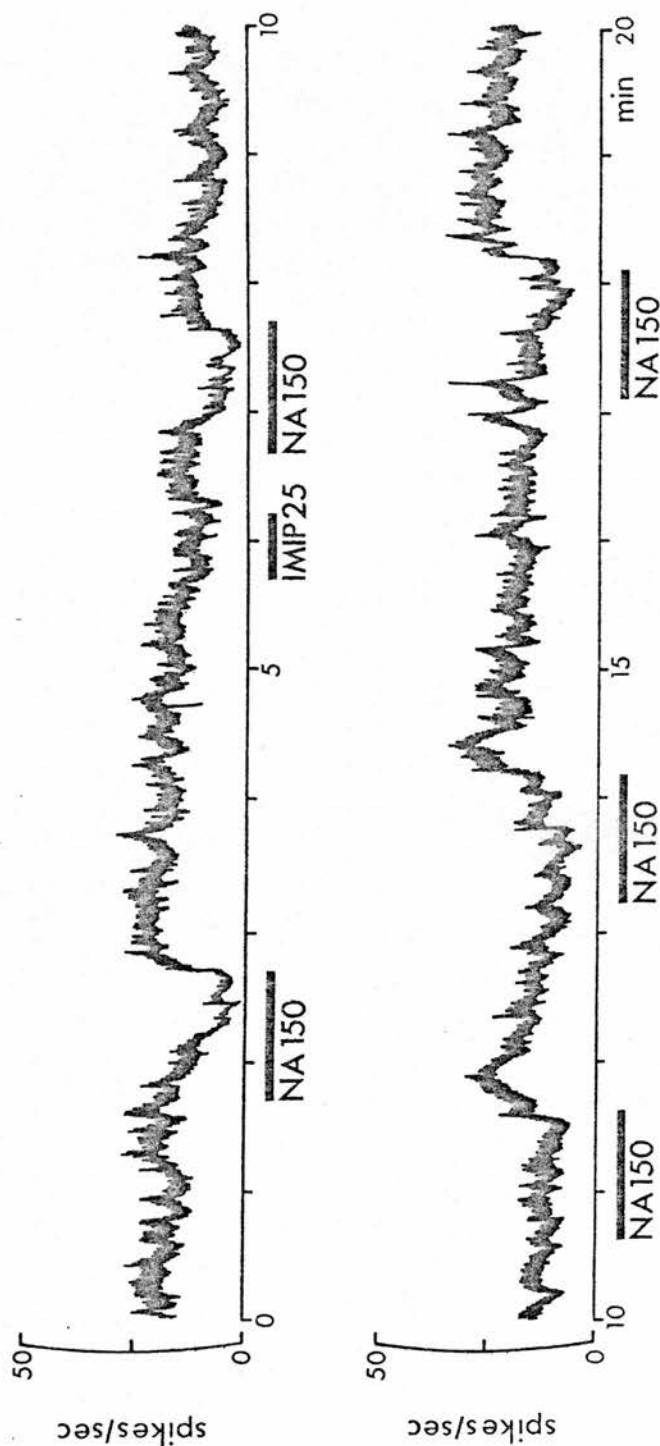


FIGURE 7/VIII. Effect of imipramine on depressant responses to noradrenaline.

Continuous ratemeter recording of the firing rate of a single cortical neurone in the cat. Ordinates: firing rate (spikes/sec); abscissae: running time (min). Horizontal bars indicate micro-electrophoretic drug applications; numbers refer to the intensities of ejecting currents (nA).

Noradrenaline (NA) (50 nA for 1 min) depressed the neurone. Imipramine (Imip) (25 nA for 30 sec) did not affect the firing rate. After the application of imipramine, the depressant response to noradrenaline was reversed into an excitation; later the depressant response re-appeared.

#### VIII.4. DISCUSSION

Imipramine and desipramine, when applied for a brief period of time, did not have any effect on the firing rates of the vast majority of cortical neurones (see Table 1/VIII). On a small number of cells, however, a response to imipramine or desipramine could be observed. A possible explanation for this effect of the antidepressants themselves could be that it reflects the interaction between endogenously released NA and the antidepressants. As responses to 5-HT (Bradshaw et al., 1974) and ACh (Bevan et al., 1975a) are also affected by imipramine and desipramine, an interaction with 5-HT and ACh, released by serotonergic and cholinergic terminals, respectively, should also be considered. On a few cells, a reduction in spike amplitude could be observed in response to either of the antidepressants. This probably reflects the local anaesthetic action of these drugs (Domenjoz & Theobald, 1959).

We have found that imipramine and desipramine have a dual effect on responses of cerebral cortical neurones to NA: both potentiation and antagonism of the responses can be observed after a brief application of either of the antidepressants. Similarly, Avanzino et al. (1971) have reported that imipramine can both potentiate and antagonise neuronal responses to NA in the brain stem. On the other hand, only potentiation of responses to NA by desipramine has been described on cerebellar Purkinje cells (Hoffer et al., 1971b).

The dual effects of the antidepressants occurred according to a dose-dependent pattern in our experiments: when different doses of the same antidepressant were compared on the same cells, smaller doses potentiated, and bigger doses antagonised the neuronal responses to NA (see Figs. 3/VIII and 4/VIII). This dual effect can be interpreted in terms of two independent mechanisms: a more sensitive potentiating mechanism, and a less sensitive antagonising mechanism. The pattern of occurrence of both potentiation and antagonism in our experiments may be explicable in terms of hypothetical concentration changes following a brief application of the antidepressant (Fig. 8/VIII). After the brief ejecting pulse the concentration of the antidepressant probably rises quickly to a peak, and then gradually declines (Castillo & Katz, 1955). When a relatively small dose of antidepressant is applied, (curve 1 in Fig. 8/VIII) the concentration rises fast above the hypothetical threshold for the potentiating mechanism (P), but it does not reach the higher threshold for the antagonising mechanism (A). Thus potentiation of the response to NA is observed. The first response after the antidepressant, which coincides with the highest antidepressant concentration, will show the greatest degree of potentiation ('early potentiation'). When a slightly bigger dose of antidepressant is applied (curve 2 in Fig. 8/VIII), the

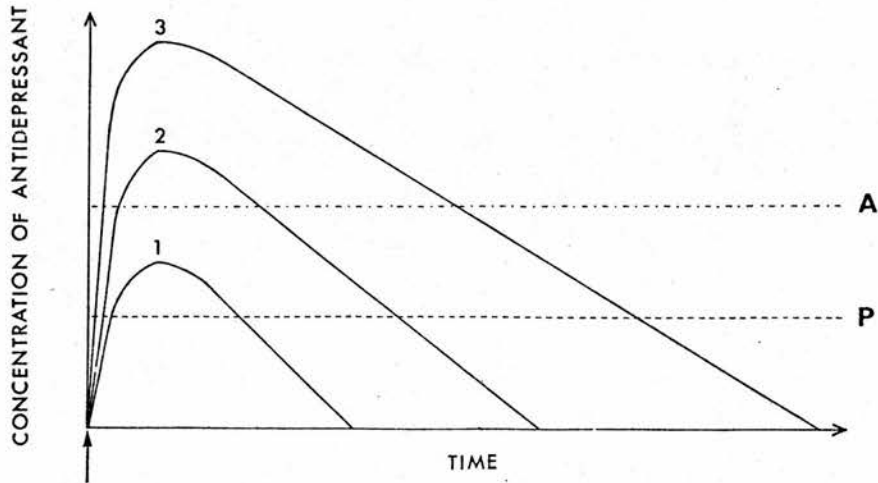


FIGURE 8/VIII. Hypothetical concentration changes following a brief application of an antidepressant.

Horizontal lines show the threshold for the potentiating (P) and the antagonising (A) mechanisms (see text). Curve 1, 2, 3: concentration curves following small, intermediated and large doses of the antidepressant.

concentration may rise slightly above the antagonism threshold (A). Therefore, at the peak of the concentration curve the size of the response to NA will reflect the relationship between the activation of the two mechanisms. Thus a smaller degree of potentiation may be followed by a greater degree of potentiation as the concentration falls below the antagonism threshold ('late potentiation'). If a still bigger dose is applied (curve 3 in Fig. 8/VIII), the concentration of the antidepressant rises high above the antagonism threshold. Thus the response to NA may be first reduced in size, and potentiation develops later, as the concentration of the antidepressant falls (antagonism followed by potentiation). The occurrence of antagonism followed by recovery (but by no potentiation), does not fit readily into this model. A possible explanation is that the study was not continued for long enough after the appearance of recovery, since this recovery occurred very often after a longer delay (up to 90-100 min). Another possibility is that in these cases the existence of a relatively higher concentration of the antidepressant for a longer time results in a persistent binding of the antidepressant to the receptors, thus interfering with the appearance of potentiation when the concentration of the antidepressant falls below the antagonism threshold.

The dual effect of the antidepressants on responses to NA may reflect the separate presynaptic and post-synaptic actions of these drugs. Potentialiation of neuronal responses to NA by imipramine and desipramine may reflect the blockade of uptake of NA into pre-synaptic terminals. It has been demonstrated that there is a powerful uptake mechanism for NA in the brain, and that this mechanism can be blocked by imipramine and desipramine (Glowinski & Axelrod, 1964; Ross & Renyi, 1967). Furthermore, it has been reported that, after pre-treatment with 6-hydroxydopamine, desipramine fails to potentiate responses of cerebellar Purkinje cells to NA (Hoffer et al., 1971b). As 6-hydroxydopamine destroys the NA-containing neurones in the brain (Bloom et al., 1969), this observation could indicate the importance of NA-containing terminals for the potentiation of neuronal responses to exogenously applied NA. Antagonism of neuronal responses to NA, on the other hand, may be due to the blockade of post-synaptic NA-receptors by the tricyclic antidepressants. The antagonism of excitatory responses to NA, which are mediated by  $\alpha$ -adrenoceptors (see Chapter V), probably reflects the well-documented  $\alpha$ -adrenoceptor blocking properties of imipramine and desipramine (Callingham, 1966; Sturman, 1971; McCulloch & Story, 1972). The antagonism of depressant responses to NA, which are mediated by  $\beta$ -adrenoceptors, would suggest that imipramine and desipramine may be able to block  $\beta$ -adrenoceptors as well. Indeed, recent evidence indicates that the number

of  $\beta$ -receptors in the brain decreases after chronic pre-treatment with desipramine (Banerjee et al., 1977); this observation could indicate an irreversible  $\beta$ -adrenoceptor blocking action of the antidepressant (see Furchgott, 1972; Triggle & Triggle, 1976). Furthermore, there is evidence in the periphery which suggests that imipramine and desipramine may have  $\beta$ -adrenoceptor blocking properties (e.g. Govier, 1967; Tamayo et al., 1973; Scriabine, 1969).

On one cell, imipramine reversed the depressant response into an excitation (Fig. 7/VIII). This observation would suggest that the inhibitory ( $\beta$ ) receptors are more sensitive to the antidepressants than the excitatory ( $\alpha$ ) receptors: the selective blockade of the dominant inhibitory  $\beta$ -adrenoceptors may have resulted in the unmasking of the excitatory effect mediated by the  $\alpha$ -adrenoceptors.

The tricyclic antidepressants imipramine and desipramine can modify not only neuronal responses to NA, but also neuronal responses to 5-HT and ACh: both potentiation and antagonism of these responses has been described (Bradshaw et al., 1974; Bevan et al., 1975a). The antidepressants are specific, however, in that responses to glutamate are not affected (VIII.3.3.). This would suggest that imipramine and desipramine do not interfere with the basic machinery of spike initiation.



CHAPTER IX

EFFECT OF IPRINDOLE ON NEURONAL RESPONSES TO NORADRENALINE

### IX.1. INTRODUCTION

According to the monoamine theory of affective disorders, the tricyclic antidepressant drugs exert their antidepressant effects by potentiating the pharmacological actions of NA at post-synaptic receptor sites in the brain (Schildkraut, 1965; Davis, 1970). It is generally believed that the NA-potentiating effect of the tricyclic antidepressants is due to their ability to block the uptake of NA into NA-containing nerve terminals (Iversen, 1974). Previous reports on imipramine and desipramine seem to confirm this hypothesis. It has been shown that these antidepressants block the uptake of noradrenaline (NA) into brain tissue (Ross & Renyi, 1967), and that they also potentiate neuronal responses to NA (see Chapter VIII). There are, however, reports suggesting that the 'uptake blockade hypothesis' of potentiation may not apply to another antidepressant, iprindole.

Iprindole is a tricyclic antidepressant drug with a similar structure and clinical antidepressant efficacy to imipramine (Imlah et al., 1968; Rickels et al., 1973). Similarly to imipramine, iprindole potentiates the peripheral effects of NA (Gluckman & Baum, 1969). However, in contrast to imipramine and other tricyclic antidepressant compounds, iprindole does not block the uptake of NA into sympathetically innervated tissues (Gluckman & Baum, 1969; Lahti & Maickel, 1971).

It has also been reported that, in contrast to imipramine and desipramine, iprindole is ineffective in blocking the uptake of NA (Ross, Renyi & Ögren, 1971; Rosloff & Davis, 1974) into brain tissue. Therefore, it was of interest to examine how pharmacological responses to NA are affected by iprindole in the brain.

#### IX.2. METHODS

All the experiments described in this Chapter were conducted in rats. Our methods for the preparation of the animals have been described in IV.2. For the methods of manufacturing six-barrelled glass micropipettes see II.2. and III.2.2.; for the methods of recording action potentials and applying drugs by microelectrophoresis see III.2.3. and III.2.4.

The micropipettes were filled with the following drug solutions: noradrenaline bitartrate (0.1 M, pH 3.0-3.5), dopamine hydrochloride (0.1 M, pH 4.0-4.5), 5-hydroxytryptamine bimaleate (0.1 M, pH 3.5), acetylcholine chloride (0.1 M, pH 3.6), sodium glutamate (0.05 M, pH adjusted to 8.5 by the addition of 0.1 M NaOH), and iprindole hydrochloride (0.1 M, pH 4.6).

All the neurones studied were spontaneously active. All the drugs were applied by microelectrophoresis. Repeated responses to an agonist were compared before and after a brief application of iprindole. In order to ensure that standard ejecting current pulses gave rise to standard pulses of drug ejection, the intervals between drug applications were kept constant using a sequential timing device (see II.3.3. and III.3.2.). A retaining current of 25 nA was passed between drug ejections. Cells were excluded from drug interaction studies if the variation in the size of responses to an agonist exceeded  $\pm 10\%$ .

The magnitude of a response was measured by calculating the difference between the number of spikes generated during the response and the number of spikes generated during an equivalent period when no drug was applied ('total spike number'; see III.2.5.).

### IX.3. RESULTS

#### IX.3.1. DIRECT EFFECT OF IPRINDOLE ON NEURONAL FIRING

The effect of iprindole on the firing rate was studied on 68 cells. The dose of iprindole applied was 30-100 nA passed for 30-60 seconds. Four cells (5.9% of

the total population of 68) responded with a clear increase in firing rate to the application of iprindole, whereas 18 cells (26.5%) were depressed (for examples see Fig. 1/IX). There was no significant correlation between the dose of iprindole applied and the effect on neuronal firing. The excitation or depression was always of a temporary nature, and the original baseline firing rate recovered within 1-3 minutes after the application of iprindole had been terminated. On occasions a reduction in spike amplitude was observed; such cells were not used for drug interaction studies.

### IX.3.2. EFFECT OF IPRINDOLE ON NEURONAL RESPONSES TO NORADRENALINE

#### a. Excitatory responses

Both potentiation and antagonism of excitatory responses to NA could be observed after a brief application (30-100 nA for 30-60 seconds) of iprindole.

Potentiation was seen on 6 cells. A response was regarded as potentiated if there was more than 20% increase over the size of the mean control response (see VIII.3.2.). An example of potentiation is shown in Fig. 2/IX. On one cell, the response was first reduced in size following the application of iprindole; this antagonism was followed later by potentiation, and finally by recovery of the control

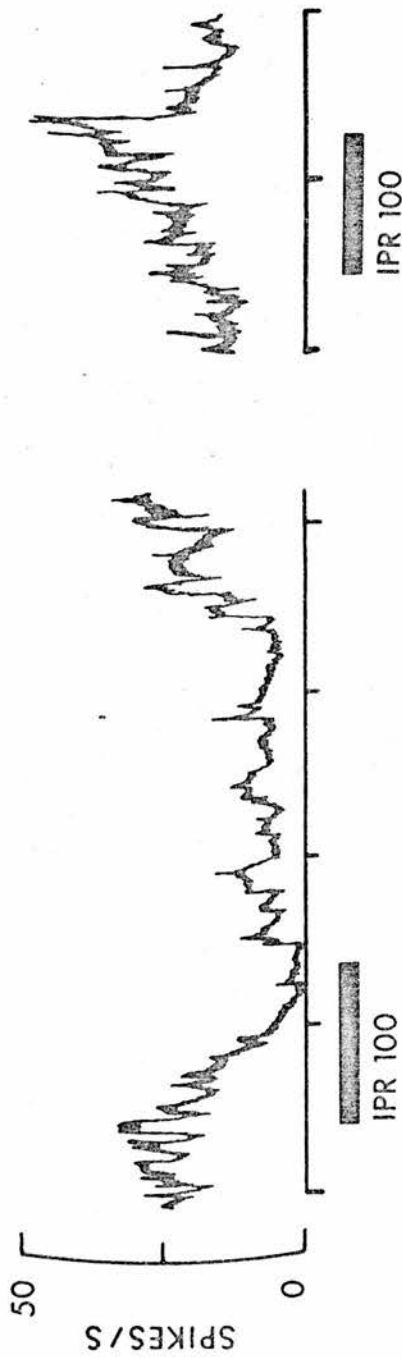
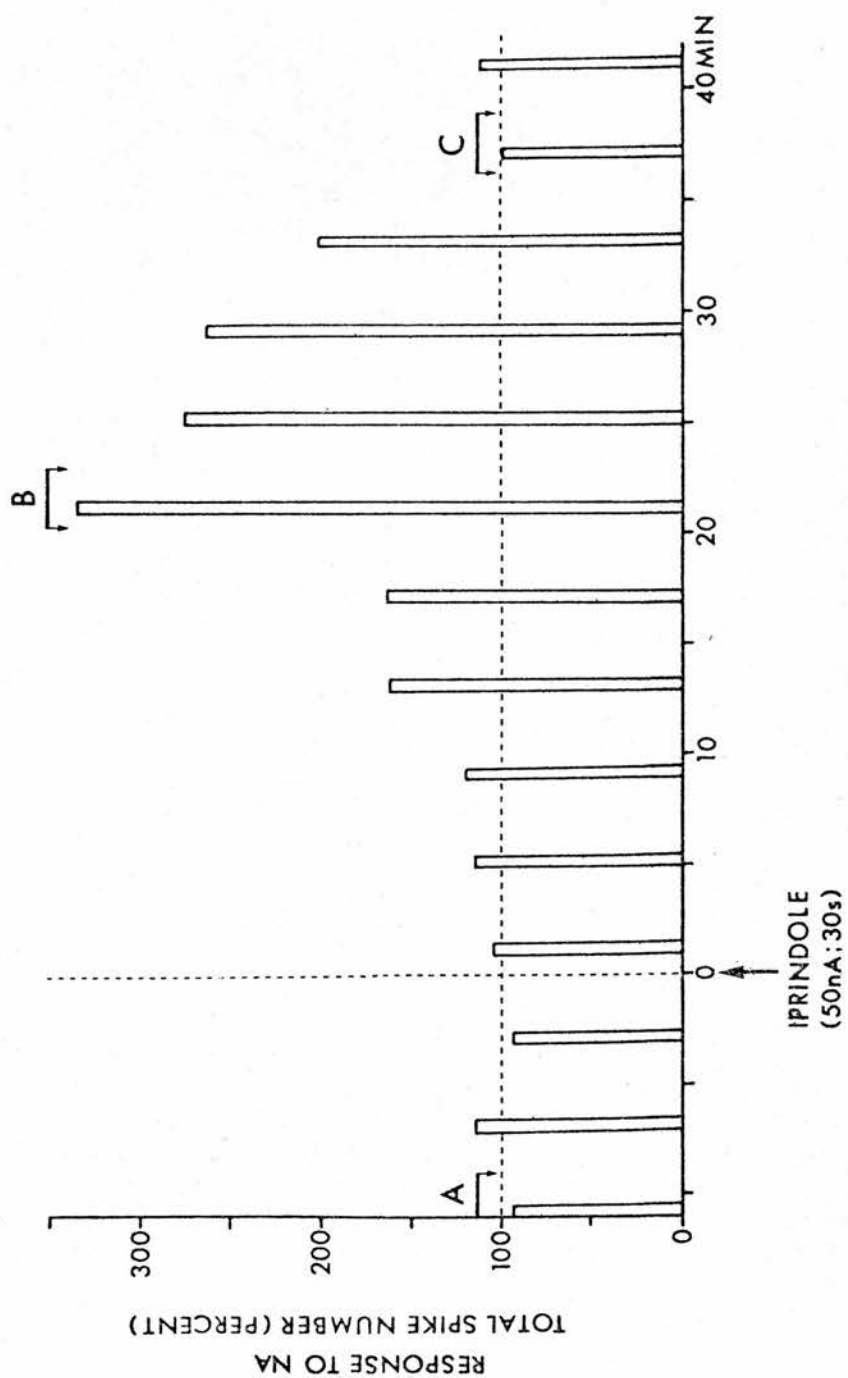
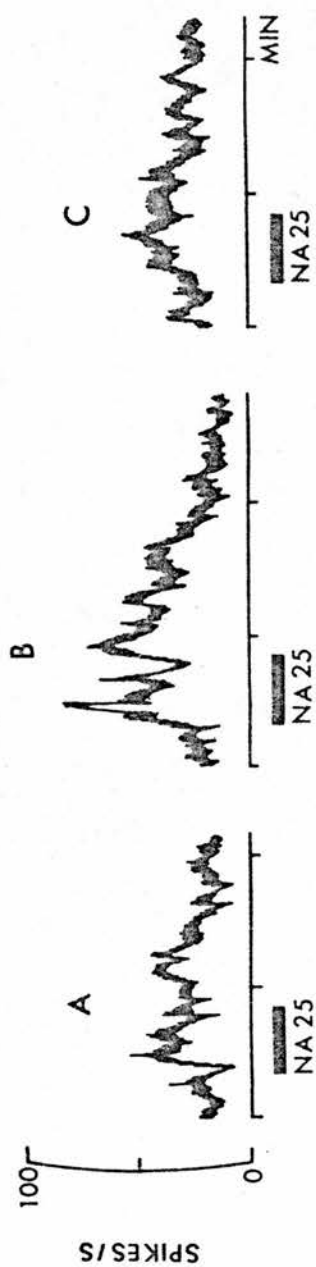


FIGURE 1/IX. Examples of responses of cortical neurones to iprindole  
Rate-meter recordings of the firing rates of two cortical neurones in the rat. Ordinate: firing rate (spikes/sec); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents (nA).  
Left: depressant response to iprindole; right: excitatory response to iprindole.



response. (A response was regarded as antagonised if there was more than 20% decrease in the size of the response compared to the mean of the control responses.)

#### b. Depressant responses

Both potentiation and antagonism of depressant responses to NA could be observed after a brief application of iprindole. Potentiation was seen on 10 cells, antagonism was seen on 3 cells. On 2 cells both antagonism and potentiation could be observed; in both cases antagonism preceded potentiation. An example of the potentiation of the depressant response to NA by iprindole is shown in Fig. 3/IX.

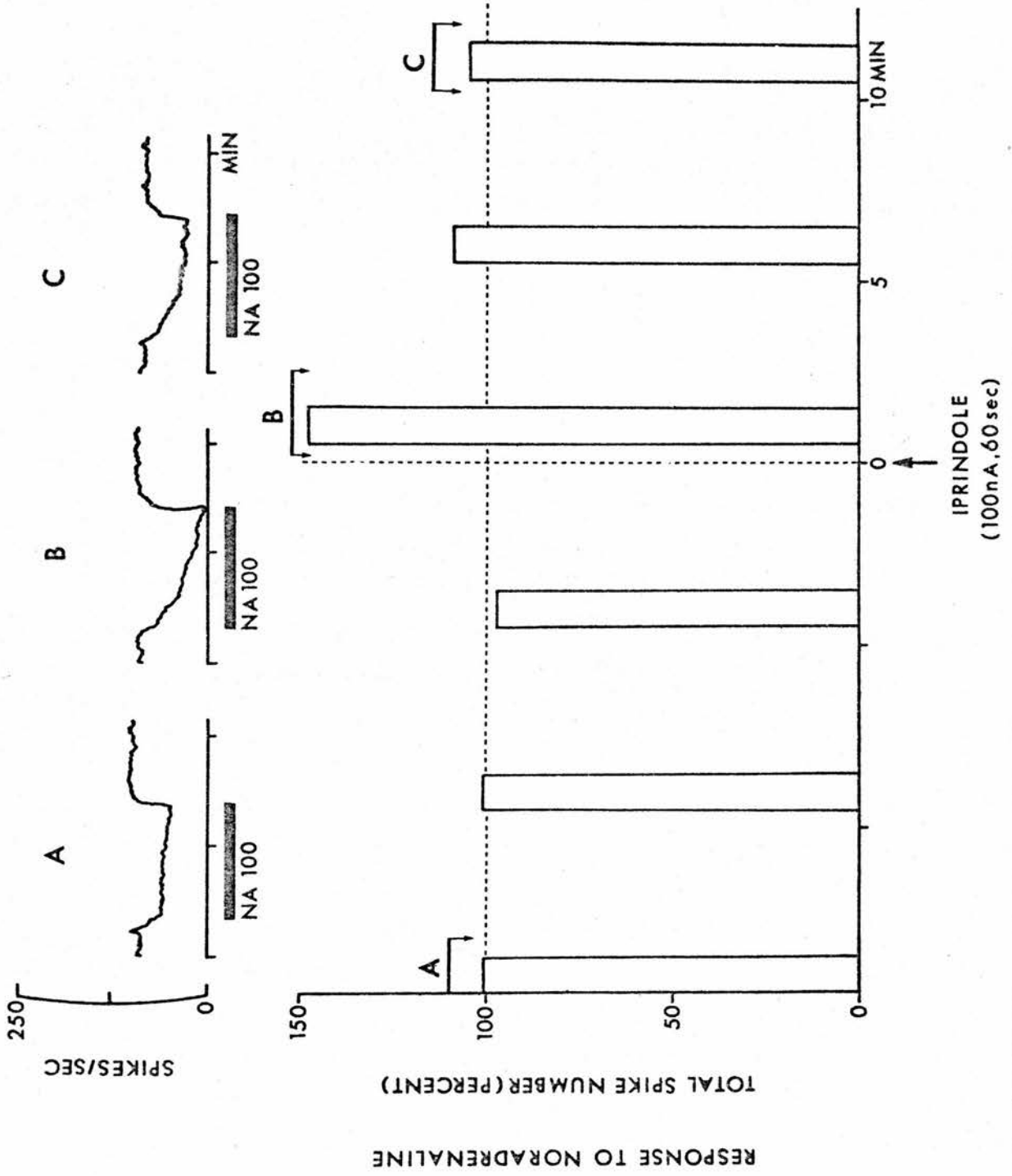
#### IX.3.3. EFFECT OF IPRINDOLE ON NEURONAL RESPONSES TO GLUTAMATE

The effect of iprindole on excitatory responses to glutamate was studied on 10 cells. The effects of glutamate on the firing rate were studied for 20-30 minutes following the application of iprindole. On none of the cells could any significant change be observed in the size of the response to glutamate after the application of iprindole.

#### IX.4. DISCUSSION

A brief application of iprindole did not affect the firing rate of the majority of cortical neurones tested.





On a third of the cells (32.4%), however, a response to iprindole was observed. A possible explanation for this effect of iprindole itself could be that it reflects the interaction between endogenously released neurotransmitters and the antidepressant. Similarly to iprindole, imipramine and desipramine can also evoke both excitatory and depressant responses on spontaneously firing cortical neurones (see VIII.3.1.). On a few cells, a reduction in spike amplitude was observed in response to iprindole. This probably reflects a local anaesthetic action of the drug. A similar local anaesthetic action has been observed with other tricyclic antidepressants (see VIII.3.1. and Domenjoz & Theobald, 1959).

We have found that iprindole, similarly to imipramine and desipramine (see Chapter VIII) can cause both antagonism and potentiation of responses of cortical neurones to NA. When both antagonism and potentiation of the response occurred after a single application of iprindole, antagonism preceded the development of potentiation. After a brief ejecting pulse, the concentration of iprindole probably rises quickly to a peak, and then gradually declines, so that antagonism of the response to NA is likely to reflect a higher, and potentiation a lower concentration of the antidepressant (see VIII.4.).

The most plausible explanation for antagonism is the blockade of NA-receptors on the post-synaptic neurone. This would indicate that iprindole probably shares the adrenoceptor blocking actions of other tricyclic antidepressants (see Chapter VIII). Indeed, recent evidence suggests that iprindole, similarly to desipramine, can interact with  $\beta$ -adrenoceptors in the brain (Banerjee et al., 1977; see also VIII.4.).

It is more difficult to interpret the potentiation of neuronal responses to NA by iprindole. As iprindole is ineffective in blocking the uptake of NA into brain tissue (Ross et al., 1971), uptake blockade cannot be an explanation for the potentiation observed in our experiments. Therefore, it is necessary to consider whether potentiation could be explained by a post-synaptic mechanism.

One possibility is that not only antagonism, but also potentiation of excitatory responses to NA by iprindole is due to post-synaptic receptor blockade. If we assume that inhibitory  $\beta$ -adrenoceptors are more sensitive to the antidepressants than the excitatory  $\alpha$ -adrenoceptors, a lower concentration of the antidepressant might block the masked inhibitory receptors, without affecting the dominant excitatory receptors, thus causing potentiation of the response. Indeed,

sotalol, a  $\beta$ -adrenoceptor blocking agent can potentiate excitatory responses to NA (Bevan et al., 1974b). The selective blockade of the inhibitory receptors may explain the reversal of the depressant response into an excitatory one by imipramine (Fig. 7/VIII), and the antagonism of the small initial depressant phase of a biphasic response as potentiation of the excitatory phase develops after the application of imipramine (Fig. 1/VIII). It would be difficult, however, to interpret the potentiation of depressant responses by the blockade of masked receptors, since in this case it would be necessary to assume a greater sensitivity of the excitatory  $\alpha$ -adrenoceptors to the antidepressants.

Another possible 'post-synaptic' explanation for potentiation could be that it is due to the inhibition of the enzyme phosphodiesterase in the post-synaptic neurone. There is experimental evidence that the tricyclic antidepressants imipramine, desipramine and iprindole reduce the activity of this enzyme (Muschek & McNeill, 1971; Daly, 1975). Furthermore, it has been reported that both  $\alpha$ - and  $\beta$ -adrenoceptor stimulation in the mammalian brain can result in the activation of adenylate cyclase (see Daly, 1976). If the adenylate cyclase system plays a role in the mediation of both excitatory and depressant neuronal responses to NA, it would be expected that the inhibition of phosphodiesterase,

the enzyme responsible for the destruction of cyclic-AMP, would cause potentiation of the responses to NA. Indeed, it has been reported that the phosphodiesterase inhibitors aminophylline and papaverine (Lake et al., 1972), and ICI-63, 197 (Stone & Taylor, 1977) can potentiate depressant responses to NA on cortical neurones. (Note, however, that there are doubts concerning the specificity of this effect; Lake et al., 1972).

C H A P T E R   X

EFFECT OF DESIPRAMINE ON NEURONAL RESPONSES TO Mescaline

### X.1. INTRODUCTION

It is well known that the tricyclic antidepressant drugs imipramine and desipramine block the uptake of noradrenaline (NA) into nerve terminals (Horn et al., 1971). These drugs also potentiate pharmacological responses to NA in the periphery (Sigg. et al., 1963) and in the central nervous system (see Chapter VIII). Although it is widely believed that there is a causal relationship between uptake blockade and potentiation (Iversen, 1974), recent evidence indicates that potentiation of responses to NA can occur in situations where uptake blockade is unlikely to operate. For instance, desipramine can potentiate neuronal responses to NA and dopamine in the caudate nucleus (Bevan et al., 1975c), although it does not block the uptake of catecholamines in this structure (Horn et al., 1971). Furthermore, iprindole, a tricyclic antidepressant with little uptake-blocking activity (Ross et al., 1971) is effective in potentiating the responses of single cortical neurones to NA (see Chapter IX). In this Chapter further evidence is presented suggesting that uptake blockade is not a necessary condition for potentiation of neuronal responses to NA.

The hallucinogenic monoamine mescaline (3, 4, 5-trimethoxyphenylethylamine) can evoke responses on cortical neurones

which are similar to those evoked by noradrenaline (Bevan et al., 1974a; see also Fig. 1/IV). It is known that mescaline has an extremely low affinity for uptake mechanisms in the periphery (Iversen, 1967). Moreover, it has recently been reported that although there is a weak active uptake process accumulating mescaline into cortical synaptosomes, this process is not affected by desipramine (Bevan et al., 1977). In the present study we examined whether neuronal responses to mescaline can be potentiated by desipramine.

#### X.2. METHODS

Spontaneously active single neurones were studied in the cerebral cortices of cats and rats anaesthetised with halothane. All the drugs were applied by microelectrophoresis. Our techniques for the surgical preparation of the animals, for the extracellular recording of action potentials, and the microelectrophoretic application of drugs from six-barrelled micropipettes have been described in previous Chapters (see II.2., III.2., and IV.2.).

The micropipettes contained the following drug solutions: mescaline hydrochloride (0.05 M, pH 4.0); (-)noradrenaline bitartrate (0.05 M, pH 3.5); sodium glutamate (0.05 M, pH adjusted to 8.5 with NaOH); desipramine hydrochloride (0.15 M, pH 4.5).



Repeated responses to mescaline were compared before and after a brief application of desipramine (25-100 nA applied for 20-60 seconds). Cells were excluded if the variation between control responses exceeded  $\pm 10\%$  (see Chapter VIII). Only excitatory responses to mescaline were selected for study in these experiments. Total spike number was used as a measure of the response (see III.2.5.). Cells were excluded if the variation between control responses exceeded  $\pm 10\%$  (see Chapter VIII). Repeated responses to mescaline were compared before and after a brief application of desipramine (25-100 nA; 20-60 sec). A response was regarded as potentiated if there was an increase of 20% or more over the size of the mean control response; a response was regarded as antagonised if there was a decrease of 20% or more in the size of the response compared with the mean of the control responses (see Chapter VIII).

### X.3. RESULTS

#### X.3.1. EXPERIMENTS WITH CATS

The effect of desipramine was studied in the cat on 11 cortical neurones which responded with a clear and consistent excitation to mescaline. On 5 of these cells the response to mescaline was potentiated following an application of desipramine. On 6 cells desipramine antagonised the response

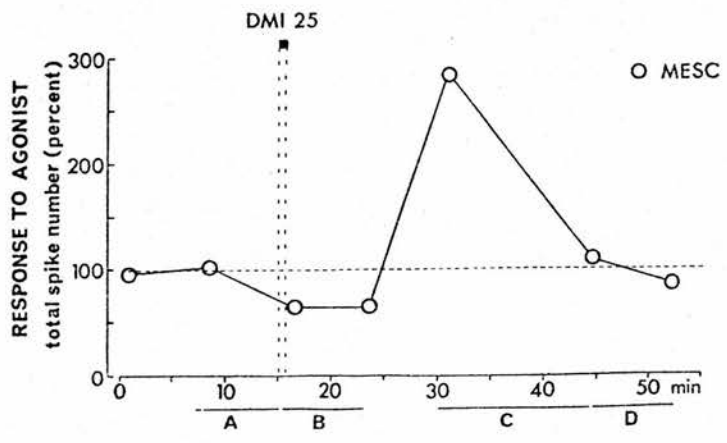
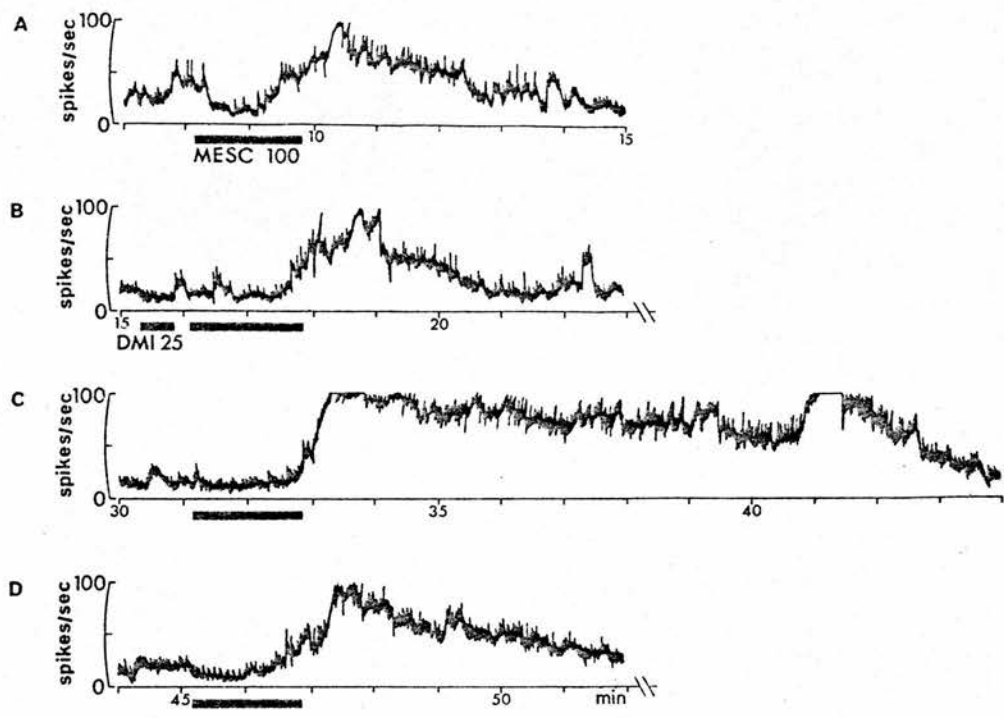
to mescaline. On 2 cells both antagonism and potentiation could be observed following a brief application of desipramine: the antagonism preceded the potentiation. An example of a study in which both potentiation and antagonism could be observed on the same cell is shown in Fig. 1/X.

#### X.3.2. EXPERIMENTS WITH RATS

Seventeen cortical neurones were studied in the rat. In these studies, glutamate was used as a control agonist. Desipramine potentiated the response to mescaline on 10 cells; on 5 of these cells the potentiation was preceded by antagonism of the response. Responses to glutamate were not affected by desipramine.

#### X.4. DISCUSSION

The effects of desipramine on responses to mescaline are similar to its effects on responses to NA (see Chapter VIII). Both antagonism and potentiation of neuronal responses to mescaline could be observed. The antagonism may reflect the  $\alpha$ -adrenolytic action of desipramine (Callingham, 1967), since mescaline and NA probably activate similar receptors on cortical neurones (Bevan et al., 1974a).



The potentiation of responses to mescaline is more difficult to explain: uptake mechanisms are unlikely to be involved, since desipramine does not block the uptake of mescaline into cortical synaptosomes (Bevan et al., 1977). An alternative possibility is that the potentiation has a post-synaptic origin: desipramine may block 'masked' inhibitory receptors on the post-synaptic neurone and thus increase the size of the observed excitatory response to mescaline, or it may inhibit the enzyme phosphodiesterase in the post-synaptic cell and thus cause potentiation of the cyclic AMP-mediated response. (For detailed discussion of these arguments see IX.4.).

C H A P T E R   X I

COMPARISON OF THE EFFECTS OF DESIPRAMINE ON NEURONAL RESPONSES  
TO TYRAMINE AND NORADRENALINE

### XI.1. INTRODUCTION

Tyramine is an indirectly acting sympathomimetic amine: it is generally believed that it exerts its pharmacological actions by the release of NA from pre-synaptic stores (Burn & Rand, 1958; Trendelenburg, 1972). It has been reported that the tricyclic antidepressant drug desipramine blocks the uptake of tyramine into sympathetically innervated tissues (Brodie et al., 1970), and thus antagonises pharmacological responses to tyramine (Gessa et al., 1966; Fozard & Mwaluko, 1976). Desipramine, however, can also block the uptake of NA (Hertting et al., 1961; Iversen, 1965), which may result in potentiation of the responses to NA (Sigg et al., 1963; Sturman, 1970). Thus desipramine, and other uptake-blocking tricyclic antidepressants (e.g. nortriptyline) can discriminate between pharmacological responses to NA and tyramine: while responses to NA are potentiated, responses to tyramine are antagonised by the same concentration of the antidepressant (Barnett et al., 1968; Fozard & Mwaluko, 1976; Doggrell & Woodruff, 1977).

The results in Chapter VIII show that desipramine can potentiate neuronal responses to NA in the brain. The experiments described in this Chapter were performed in order to 1. compare the agonistic activities of tyramine and NA on cortical neurones, and 2. examine whether desipramine can discriminate between neuronal responses to tyramine and NA.

## XI.2. METHODS

### XI.2.1. PHARMACOLOGICAL EXPERIMENTS

Male albino Wistar rats (250-350g) were used in these experiments. The animals were anaesthetised with halothane (0.8-1.0%). Our methods for the surgical preparation of the animals, for the manufacture of six-barrelled micropipettes, for the extracellular recording of action potentials, and for the microelectrophoretic application of drugs have been described in previous Chapters (II.2., III.2. and IV.2.).

Six-barrelled micropipettes of tip diameter 3.0-5.0  $\mu\text{m}$  were used. Two barrels of each micropipette contained 4 M NaCl, one for recording action potentials, the other for use in current balancing. The remaining barrels contained drug solutions. The following drug solutions were used; tyramine hydrochloride (0.05 M, pH 5.0); (-)noradrenaline bitartrate (0.05 M, pH 3.0-3.5); DL-homocysteic acid (0.05 M, pH adjusted to 8.0 with NaOH); desipramine hydrochloride (0.005 M, pH 4.5).

Spontaneously active neurones were studied in the cerebral cortex (stereotaxic co-ordinates, according to König & Klippel (1963): A 4.8-6.5, L 0.9-2.4). The area of recording was prepared as described previously (see III.2.1.). The dura was either incised with a hypo-

dermic needle, or was penetrated directly with the micropipette. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive applications of agonists retaining currents of  $-10$  nA were passed. Retaining currents of  $-25$  nA were used for desipramine. Intervals between successive applications of the same agonist were kept constant in order to standardise the effects of the retaining current upon drug release during the ejection period (see II.3.3., III.3.2.).

Two measures of the overall size of the neuronal response were used in these experiments (see III.2.5.). Total spike number was used when changes in the size of the response to a particular agonist were assessed. Total spike number is the total number of action potentials produced in response to each drug application; this measure is proportional to the area enclosed by the response curve. Maximum change in firing rate was used when responses to different agonists were compared, e.g. when assessing the relative potencies of tyramine and NA.

The effect of desipramine was evaluated in the following way. When stable responses to the agonists had been obtained, desipramine was applied continuously, either by removal of the retaining current (i.e.  $0$  nA) thus allowing the drug to diffuse out from the micropipette, or by the



passage of a weak ejecting current (5 to 10 nA), and the time-course of the developing antagonism or potentiation was followed. If necessary, the intensity of the ejecting current was increased until antagonism or potentiation was observed. Then the application of desipramine was terminated, and the time-course of recovery of the responses to the agonist(s) was followed.

#### XI.2.2. MEASUREMENT OF THE RELEASE OF TYRAMINE AND NORADRENALINE FROM MICROPIPETTES IN VITRO

Six-barrelled micropipettes were used in these experiments. Three barrels of each micropipette were filled with 0.05 M [sidechain-2-<sup>14</sup>C] tyramine; hydrochloride; the remaining three barrels contained 0.05 M [carbinol-<sup>14</sup>C] noradrenaline bitartrate (Radiochemical Centre, Amersham). The specific activities of both solutions were 1.0 mCi/mmole. Our methods for the collection of samples, and for the calculation of transport numbers have been described in II.2. and VI.2.2.

### XI.3. RESULTS

#### XI.3.1. COMPARISON OF AGONISTIC EFFECTS OF TYRAMINE AND NORADRENALINE

##### a. Responses of cortical neurones to tyramine

Both excitatory and depressant responses to tyramine were observed in these experiments. Out of 165 cells

responding to tyramine, 111 cells (67.3%) were excited, and 54 cells (32.7%) were depressed by the drug. The predominance of excitatory responses is statistically significant (binomial test;  $p < 0.01$ ).

b. Comparison of directions of responses to tyramine and NA

Each of the 165 cells responding to tyramine (see above), responded also to NA. The correlation between the directions of the responses (excitation or depression) is summarized in Table 1/XI. It is apparent from the table that the great majority of the cells responded in the same direction to the two drugs, cells excited by tyramine being also excited by NA, and cells depressed by tyramine being also depressed by NA. This positive correlation between the effects of tyramine and NA is statistically significant ( $\chi^2$  test;  $p < 0.001$ ). Examples of the cells responding in the same directions to the two drugs are shown in Fig. 1/XI.

c. Comparison of apparent potencies of tyramine and NA

The relative potencies of tyramine and NA were assessed in terms of the equipotent current ratio. The equipotent current ratio was defined as the ratio of ejecting currents (current for tyramine/current for NA)

TABLE 1/XI. Correlation between the effects of tyramine and noradrenaline.

		TYRAMINE	
		+	-
NORADRENALINE	+	110	2
	-	1	52

n = 165

$\chi^2$  test;  $p < 0.001$

+ : excitatory responses; - : depressant responses.

Numbers: number of cells responding to both tyramine and noradrenaline.

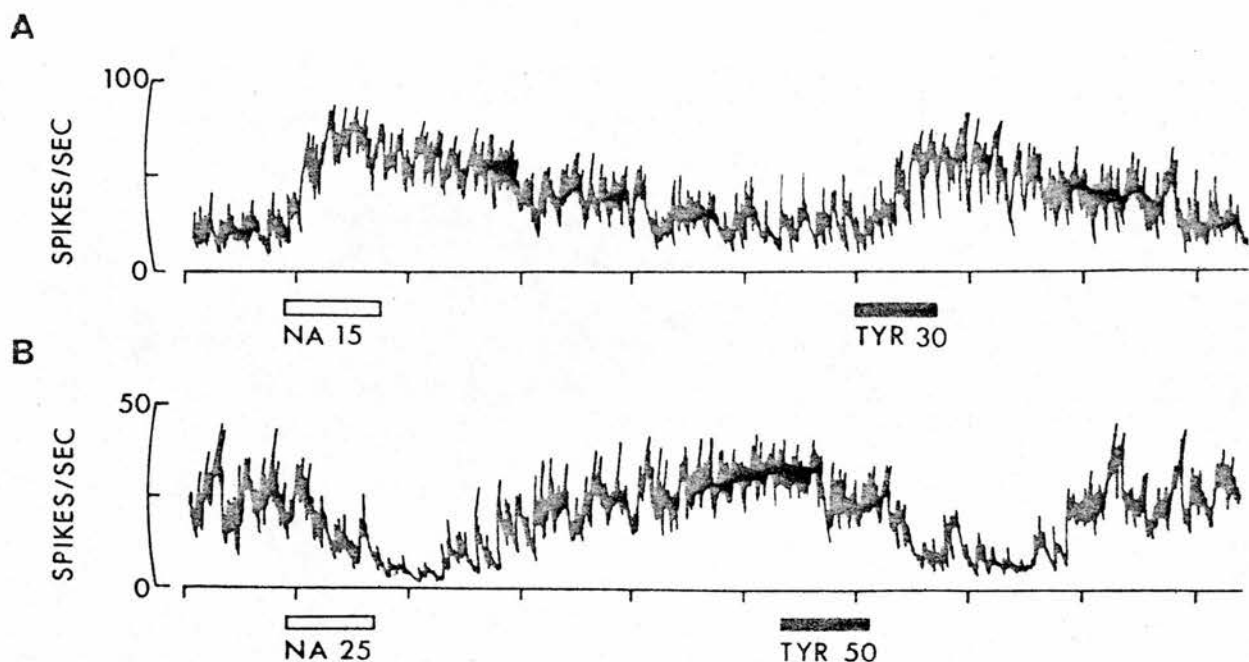


FIGURE 1/XI. Correlation between the effects of tyramine and noradrenaline.

Ratemeter recordings of the firing rates of two cortical neurones in the rat (A and B). Ordinates: firing rate (spikes/sec); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting current (nA).

A: A cell which was excited by both tyramine (TYR) and noradrenaline (NA).

B: A cell which was depressed by both tyramine and noradrenaline.

needed in order to evoke responses of approximately equal magnitude (maximum changes in firing rate not differing by more than 20%) to the two amines.

The relative potencies of the two drugs were compared on 67 cells (44 cells excited by both drugs, 23 cells depressed by both drugs). On each cell, in the case of both excitatory and depressant responses, tyramine appeared to be less potent than NA: the mean equipotent current ratio ( $\pm$  s.e.m.) was  $3.02 \pm 0.22$  for excitatory responses, and  $2.52 \pm 0.32$  for depressant responses. In the case of both excitatory and depressant responses, the potency difference between the two amines was statistically significant (t test;  $p < 0.001$ ).

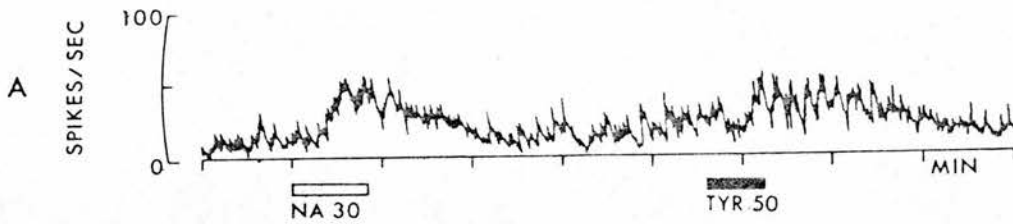
Examples of the potency comparisons are shown in Fig. 2/XI.

#### d. Comparison of the time-course of responses to tyramine and NA

Two time-course parameters were measured: latency to onset and recovery time. Latency to onset is the time elapsed between the beginning of the ejecting pulse and the onset of the neuronal response; recovery time is the time elapsed between the termination of the ejecting pulse and the offset of the neuronal response (see III.2.5.).

These parameters were measured on 67 cells giving approximately equivalent responses (in terms of the maximum changes in

### EXCITATORY RESPONSES



### DEPRESSANT RESPONSES

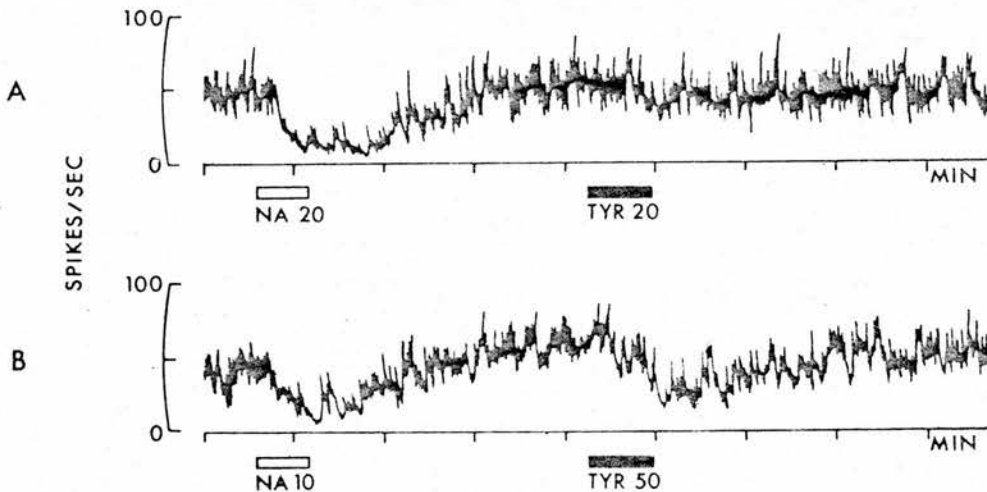


FIGURE 2/XI. Comparison of apparent potencies of tyramine and noradrenaline.

Ratemeter recordings of the firing rates of two cortical neurones in the rat (one neurone was excited, the other neurone was depressed by tyramine and noradrenaline). Ordinates: firing rate (spikes/sec); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting current (nA).

Excitatory responses: In order to evoke approximately equivalent responses, a higher current was needed to apply tyramine (TYR) than noradrenaline (NA).

Depressant responses:

A: Tyramine evoked a smaller response than noradrenaline when both drugs were applied with identical ejecting currents.

B: In order to evoke approximately equivalent responses, a higher current was needed to apply tyramine than noradrenaline.

firing rate; see above) to the two amines. Of the 67 cells, 44 cells were excited, and 23 cells were depressed by both drugs. The mean ratios of the time-course parameters are shown in Table 2/XI. These ratios were obtained by dividing the value of the parameter in the case of the response to tyramine by the value of the parameter in the case of the response to NA. It is apparent from the table that the values of these ratios were greater than one indicating that tyramine evoked 'slower' responses than NA. Examples of this finding are shown in Fig. 3/XI.

#### XI.3.2. EFFECTS OF DESIPRAMINE ON NEURONAL RESPONSES TO TYRAMINE AND NORADRENALINE

Desipramine, applied continuously from a dilute solution (0.005 M) with a low ejecting current (0-10 nA), usually did not have any effect on the firing of the neurone. On some cells, however, a decrease in firing rate occurred, or the spike amplitude was reduced. Such cells were not used for drug-interaction studies.

##### a. Excitatory responses

Drug-interaction studies were successfully completed on 47 cortical neurones excited by both tyramine and NA. DL-homocysteic acid was used as a control agonist. On 41 cells, desipramine could discriminate between responses

Table 2/XI. Ratios of the parameters of the time-courses of neuronal responses to tyramine and noradrenaline

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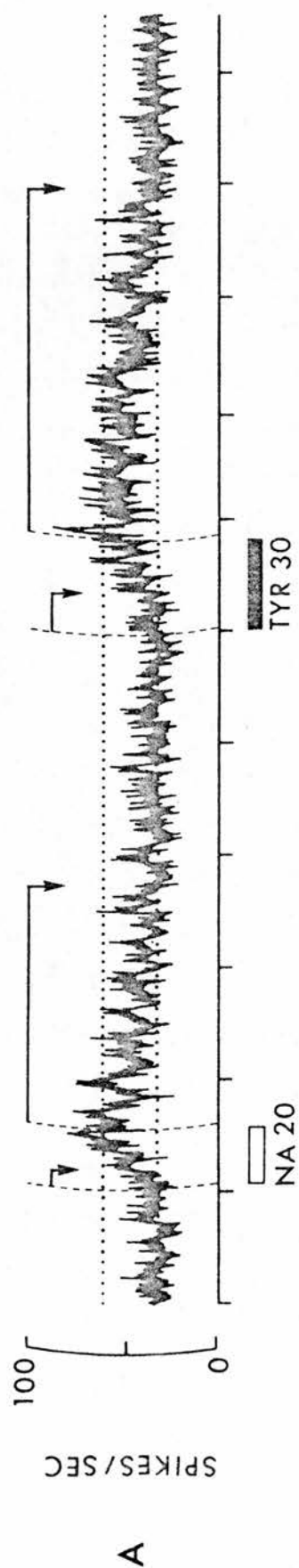
RATIO OF TIME-COURSE PARAMETERS		
(TYR/NA): mean $\pm$ s.e.m.		
<hr/>		
PARAMETER	EXCITATORY RESPONSES (n = 44)	DEPRESSANT RESPONSES (n = 23)
Latency	4.84 $\pm$ 0.76 **	2.49 $\pm$ 0.49 *
Recovery time	2.25 $\pm$ 0.15 **	1.60 $\pm$ 0.16 **

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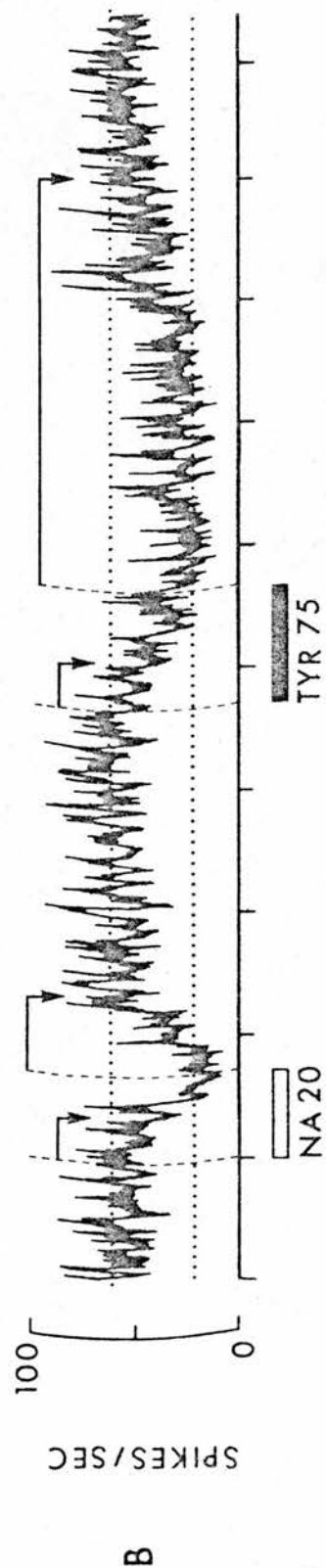
t test: \*  $p < 0.01$ ; \*\*  $p < 0.001$



## EXCITATORY RESPONSES



## DEPRESSANT RESPONSES



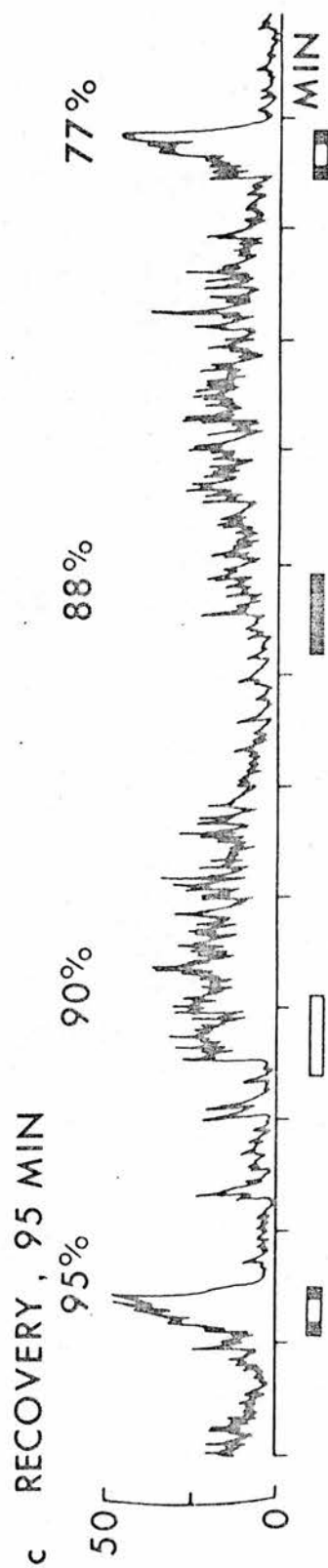
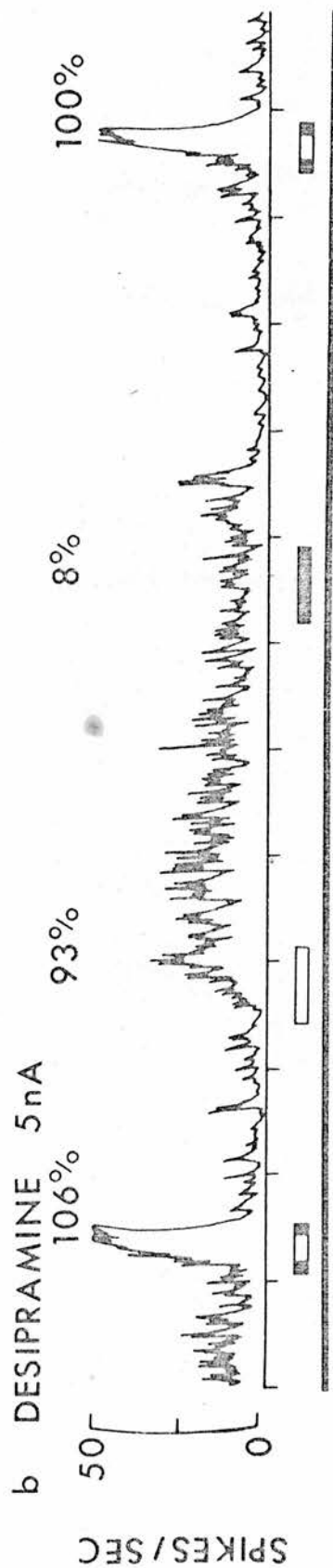
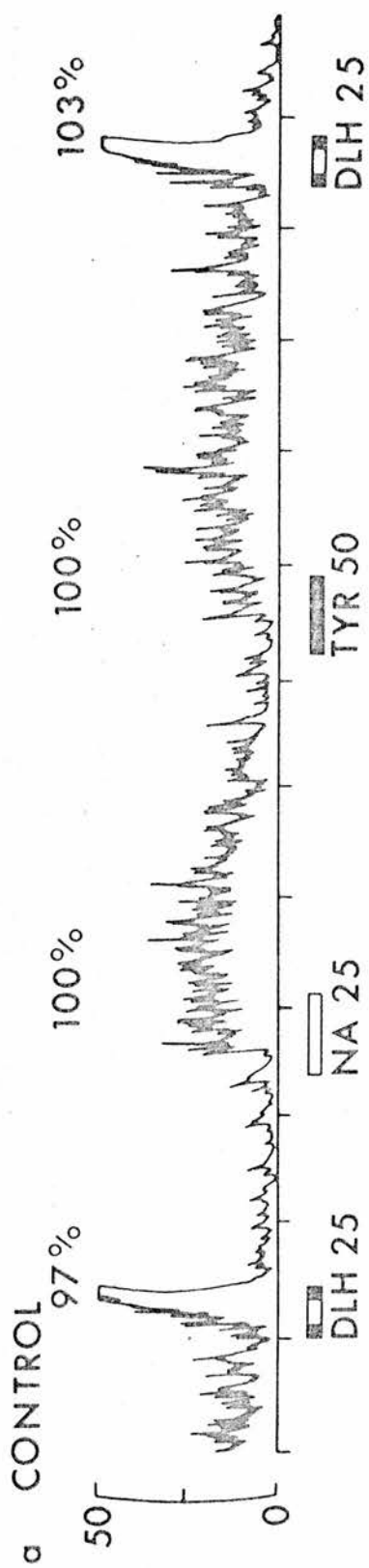
to tyramine and NA: on all these cells the response to tyramine was abolished, while the response to NA was either potentiated (11 cells) or unaffected (30 cells). On the remaining 6 neurones, the excitatory responses to tyramine and NA were equally antagonised. Responses to DL-homocysteic acid were not affected by desipramine. Recovery of responses to tyramine and NA from the effects of desipramine usually was very slow, taking on occasions as long as two hours to develop. On a few cells full recovery of the responses could not be observed even after several hours. Examples of the effects of desipramine on excitatory responses to tyramine and NA are shown in Figs. 4/XI and 5/XI.

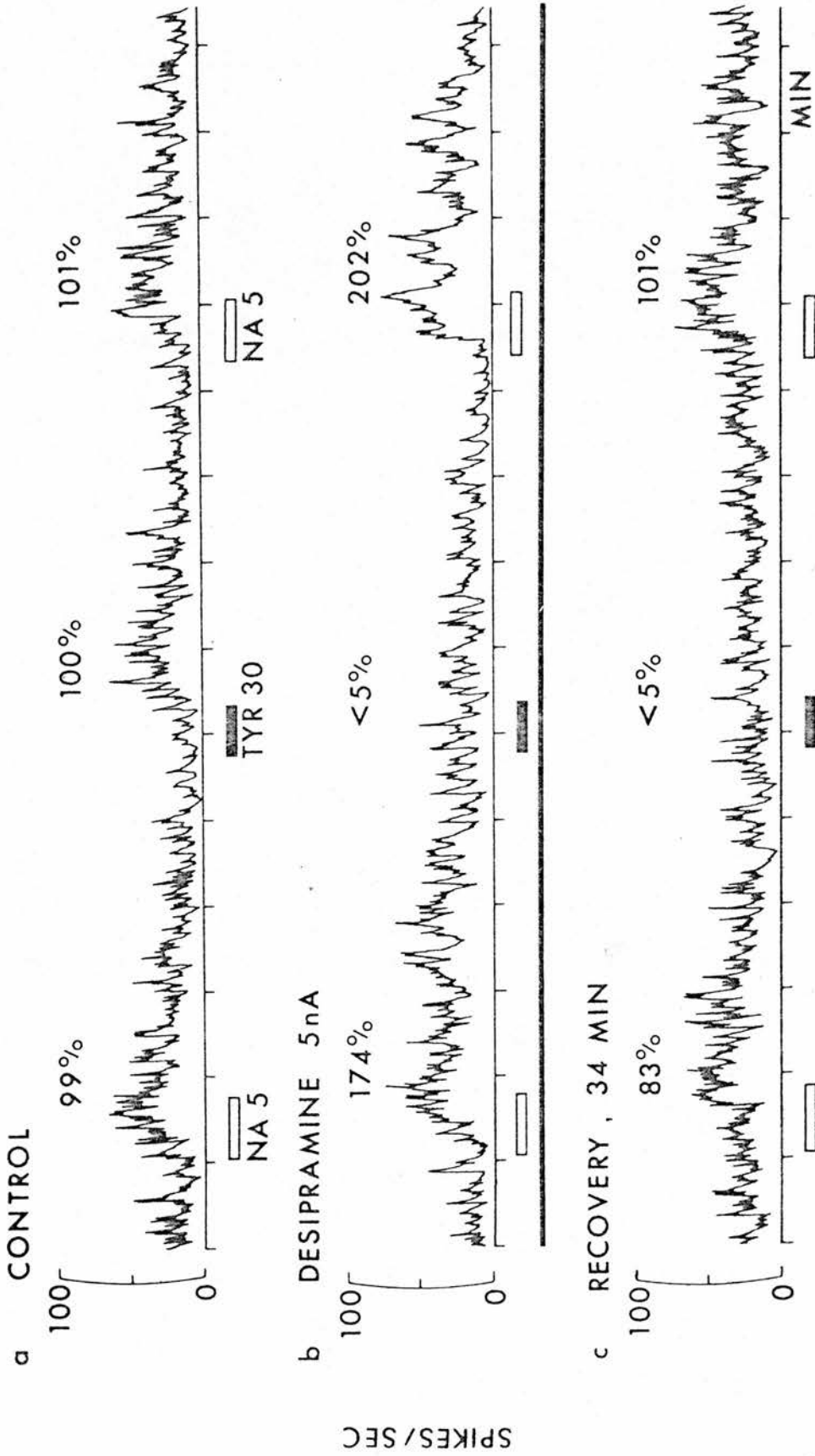
#### b. Depressant responses

The effects of desipramine were examined on 10 cells depressed by both tyramine and NA. On 8 of these cells, the response to tyramine was antagonised, while the response to NA was either potentiated (5 cells) or unaffected (3 cells). On the remaining 2 cells, responses to both amines were equally antagonised.

#### XI.3.3. COMPARISON OF THE TRANSPORT NUMBERS OF TYRAMINE AND NORADRENALINE

The transport numbers of tyramine and NA were compared using 8 micropipettes; the results obtained are





shown in Table 3/XI. There was no significant difference between the transport numbers of tyramine and NA (paired t test;  $p = 0.115$ ).

#### XI.4. DISCUSSION

There have been only a few studies of the pharmacological actions of tyramine in the brain. Hoffer et al. (1971) reported that tyramine, similarly to NA, could evoke depressant responses on cerebellar Purkinje cells. These authors suggested that tyramine might have primarily a direct post-synaptic effect in the brain, since responses to tyramine could be evoked after pretreatment with 6-hydroxydopamine, an agent which destroys NA-containing nerve terminals (Bloom et al., 1969). On the other hand, there is evidence that the machinery exists in the brain for an indirect action of tyramine: 1. tyramine is accumulated by an active uptake process into brain tissue (Ross & Renyi, 1966); 2. this uptake process is inhibited by desipramine (Steinberg & Smith, 1970); and 3. tyramine can displace NA from brain synaptosomes (Colburn & Kopin, 1972). The experiments presented in this Chapter seem to support the hypothesis that tyramine may have an indirect action in the brain.

##### XI.4.1. THE AGONISTIC EFFECT OF TYRAMINE ON CORTICAL NEURONES

Tyramine, when applied by microelectrophoresis to spontaneously active cortical neurones, could evoke both excitatory and depressant responses. There were similarities between the agonistic effects of tyramine and NA: 1. every tyramine-sensitive

TABLE 3/XI. Transport numbers of noradrenaline and tyramine obtained from eight six-barrelled micropipettes

Pipette	Tip diameter ( $\mu\text{m}$ )	Transport number (mean $\pm$ s.e.m. of 8 observations)	
		Noradrenaline	Tyramine
1	4.0	0.292 $\pm$ 0.013	0.391 $\pm$ 0.025
2	3.5	0.229 $\pm$ 0.046	0.460 $\pm$ 0.049
3	5.0	0.257 $\pm$ 0.015	0.248 $\pm$ 0.013
4	4.0	0.409 $\pm$ 0.012	0.432 $\pm$ 0.014
5	4.0	0.250 $\pm$ 0.016	0.279 $\pm$ 0.009
6	4.0	0.197 $\pm$ 0.009	0.253 $\pm$ 0.011
7	4.0	0.255 $\pm$ 0.003	0.265 $\pm$ 0.006
8	5.0	0.377 $\pm$ 0.008	0.367 $\pm$ 0.014

Paired t-test;  $p = 0.115$

cell was also sensitive to NA; 2. the vast majority of the neurones responded in the same directions to the two amines: neurones excited by tyramine being also excited by NA, and neurones depressed by tyramine being also depressed by NA.

When compared on the same neurones, tyramine appeared to have a lower potency than NA. Since there was no significant difference between the transport numbers of tyramine and NA, it can be assumed that identical ejecting currents resulted in similar rates of release of the two amines from the micropipettes (see Chapter II). It is probable, therefore, that the observed difference in potency reflects a genuine biological difference between the two drugs. The lower potency of tyramine may reflect an indirect action of the amine, since it has been reported that tyramine does not displace NA stoichimetrically from adrenergic terminals (Axelrod, et al., 1962).

The responses to tyramine had a slower time-course (longer latency and longer recovery time) than responses to NA. Such a difference between the time-courses could reflect a difference between the physical mobilities of tyramine and NA molecules (see Szabadi & Bradshaw, 1974). This is, however, not likely to have been the case in the present experiments, since the transport numbers of the two drugs were very similar, indicating similar mobilities for the tyramine and NA molecules. The slower time-course of the response to tyramine may reflect the time which is required for the tyramine molecules released from the micropipette to reach the NA-containing

nerve terminals, to be taken up by the terminals, to displace and release NA, and for the released NA molecules to reach the post-synaptic receptors.

#### XI.4.2. THE EFFECT OF DESIPRAMINE ON NEURONAL RESPONSES TO TYRAMINE

Desipramine, applied continuously from one barrel of the micropipette, could discriminate between neuronal responses to tyramine and NA: while responses to tyramine were antagonised, responses to NA were potentiated. A similar discrimination by desipramine between pharmacological responses to tyramine and NA has been reported in the periphery (Barnett et al., 1968; Fozard & Mwaluko, 1976). The most plausible explanation for this observation is the blockade of the NA-uptake mechanism by desipramine: uptake blockade would prevent tyramine from reaching the presynaptic NA stores, and thus would prevent the pharmacological response; on the other hand, uptake blockade would result in potentiation of the response to NA by interfering with the major process of removal of NA from the post-synaptic receptor sites (see Iversen, 1974). Indeed, there is evidence that desipramine blocks the uptake of both NA (Ross & Renyi, 1967) and tyramine (Ross & Renyi, 1967 ) into brain tissue.

Although potentiation of the response to NA could sometimes be observed when the response to tyramine was antagonised (see Fig. 5/XI), on many occasions no potentiation of the response to NA could be seen (see Fig. 4/XI). This observation may reflect a separate



postsynaptic action of desipramine. It is well documented that desipramine has postsynaptic adrenoceptor blocking properties in the periphery (Callingham, 1966; Sturman, 1971; McCulloch & Story, 1972). Furthermore, the experimental evidence presented in Chapter VIII shows that desipramine can antagonise responses of cortical neurones to NA. It is possible, therefore, that, in some experiments, the blockade of post-synaptic receptors by desipramine prevented the development of the potentiation of responses to NA which would have resulted from the pre-synaptic uptake blockade.

The present results are compatible with the hypothesis that there is a close relationship between uptake blockade and potentiation of neuronal responses to NA. There is evidence, however, that desipramine can potentiate neuronal responses to NA, and to other monoamines, in situations where uptake blockade is unlikely to operate. Thus responses to NA and dopamine are potentiated in the caudate nucleus (Bevan et al., 1975b) where desipramine is ineffective in blocking the uptake of catecholamines (Horn et al., 1971). Iprindole, a tricyclic antidepressant which is ineffective in blocking the uptake of NA into brain tissue (Ross et al., 1971), can potentiate neuronal responses to NA (see Chapter IX). Finally, neuronal responses to mescaline are also potentiated by desipramine (see Chapter X), although the uptake of mescaline into brain synaptosomes is not affected by desipramine (Bevan et al., 1977). These observations suggest that, although blockade may result in potentiation, its presence is not a pre-requisite for potentiation, and that there may be some postsynaptic

mechanisms which contribute to the potentiation of neuronal responses to NA by desipramine (see IX.4.).

A P P E N D I X

ANTAGONISTIC AGONISM: A THEORETICAL MODEL

### A.1. PREFACE

The experiments described in this thesis (see Chapters IV, V, VI, VII) show that noradrenaline (NA) applied by microelectrophoresis can have both excitatory and depressant effects on single cortical neurones, suggesting the presence of both excitatory and inhibitory receptors for NA. Furthermore, there is evidence indicating that both excitatory and inhibitory receptors for NA can co-exist on the same cells (see Chapters IV and V).

The review of the literature suggests that the co-existence of two functionally opposite receptor populations for the same agonist in the same pharmacological test system is a common phenomenon. The theoretical model presented in this Appendix examines the quantitative pharmacology of these 'opposite receptors'.

### A.2. INTRODUCTION

A pharmacological agonist can have either of two effects on a muscular or a neuronal test system: it can cause either an increase (i.e. excitation) or a decrease (i.e. inhibition) in the activity of the system. Pharmacological receptors mediating these effects can therefore be classified on a functional basis as excitatory or inhibitory receptors.

A special case of drug action is when the same agonist can activate both excitatory and inhibitory receptors in the same effector tissue. As the two kinds of receptor are in a functionally antagonistic relationship, the term antagonistic agonism could be used to describe this type of drug action. Dale reported in 1906 (Dale, 1906) that adrenaline can act at both excitatory and inhibitory receptors in the vascular bed, and Furchgott suggested in 1955 (Furchgott, 1955) that these receptors may occur on the same smooth muscle cell. It has emerged, however, only during the last decade that many physiologically important drugs may activate two opposing receptor populations both on smooth muscle cells and on neurones.

#### A.2.1. FUNCTIONALLY OPPOSITE RECEPTORS ON SMOOTH MUSCLE CELLS

A review of the literature reveals that most of the sympathomimetic amines can activate 'opposite receptors' in smooth muscle preparations. Adrenaline and noradrenaline act at both excitatory and inhibitory receptors in the vascular smooth muscle (Furchgott, 1955; Guimarães & Osswald, 1969), in the heart (Govier, Mosal et al., 1966; Benfey & Varma, 1967), in the gut (Guimarães, 1969; Haffner et al., 1969; Belisle & Gagnon, 1971; Haffner, 1971; Tamayo et al., 1974), in the guinea pig trachea and lung (Everitt & Cairncross, 1969; Persson & Johnson, 1970), in the mouse spleen (Ignarro &

Titus, 1968), in the cat nictitating membrane (Smith, 1963), and in the rat uterus (Diamond & Brody, 1966). Isoprenaline activates both kinds of receptor in the vascular smooth muscle (Furchgott, 1955; Guimarães & Osswald, 1969; DiSalvo & Fell, 1974), in the gut (Guimarães, 1969), in the mouse spleen (Ignarro & Titus, 1968), in the cat nictitating membrane (Trendelenburg et al., 1971; Trendelenburg, 1974), in the guinea pig vas deferens and seminal vesicle (Large, 1965; Spedding & Weetman, 1972), and in the human urinary bladder (Awad et al., 1974). Phenylephrine acts at opposite receptors in the heart (Benfey & Varma, 1967), in the gut (Guimarães, 1969; Haffner, 1971; Gagnon, 1972), and in the human urinary bladder (Awad et al., 1974). Both metaraminol and methoxamine activate both types of receptor in the gut (Gagnon, 1972). Dopamine acts at both excitatory and inhibitory receptors in the vascular smooth muscle (Goldberg, 1975). In general, the excitatory receptors are of the  $\alpha$  type, whereas the inhibitory receptors are of the  $\beta$  type (Ahlquist, 1948).

Similarly to the catecholamines, histamine can also activate both excitatory and inhibitory receptors in some tissues, such as rabbit blood vessels (Glover et al., 1973; Parsons & Owen, 1973), pulmonary vessels in the guinea pig (Okpako, 1972; Goadby & Phillips, 1973), and sheep bronchi

(Eyre, 1973). In all these tissues the excitatory receptors are of the  $H_1$  type, whereas the inhibitory receptors are of the  $H_2$  type (Black et al., 1972).

#### A.2.2. FUNCTIONALLY OPPOSITE RECEPTORS ON NERVE CELLS

##### a. Invertebrates

The existence of opposite receptors to acetylcholine has been described on abdominal ganglion cells in Aplysia (Wachtel & Kandel, 1967), on buccal ganglion cells in Aplysia (Gardner & Kandel, 1972), on pleural ganglion cells in Aplysia (Kehoe, 1972a; 1972b), and on the buccal ganglion cells of the mollusc Navanax (Levitan & Tauc, 1972; 1975). The excitatory receptor mediates an increase in sodium permeability, and can be blocked by both curare and hexamethonium (Kehoe, 1972a; 1972b; Kehoe & Marder, 1976). It has been possible to separate two inhibitory receptors which differ from each other in ionic selectivity and pharmacological specificity. One kind of inhibitory receptor mediates an increase in chloride permeability, and can be blocked by curare, but not by hexamethonium. The other kind of inhibitory receptor mediates an increase in potassium permeability, and can be selectively blocked by tetraethylammonium (TEA), but not by curare (Kehoe, 1972a; Kehoe & Marder, 1976). The sodium-dependent excitatory receptor can co-exist

with the chloride-dependent inhibitory receptor (Gardner & Kandel, 1972; Kehoe, 1972b; Levitan & Tauc, 1972; Levitan & Tauc, 1975), with the potassium-dependent inhibitory receptor (Kehoe, unpublished; see Kehoe, 1972b), or indeed with both types of inhibitory receptor (Kehoe, 1972b). The stimulation of the presynaptic neurone can evoke a biphasic synaptic potential consisting of an excitatory post-synaptic potential (EPSP) and an inhibitory post-synaptic potential (IPSP) (Gardner & Kandel, 1972; Levitan & Tauc, 1975), or an EPSP at low frequency of stimulation, which is reversed into an IPSP as the stimulation frequency is increased (Wachtel & Kandel, 1967), or a monophasic EPSP which is converted into an IPSP by hexamethonium, the specific blocker of the excitatory receptors (Kehoe, 1972b).

Dopamine can also activate both excitatory and inhibitory receptors on the same neurones in the pleural ganglion of Aplysia (Ascher, 1972; Ascher, 1973), and in the pleural ganglion of Planorbis (Berry & Cottrell, 1973; 1975). The excitatory receptor probably mediates an increase in sodium permeability (Ascher, 1973), and can be blocked by curare and strychnine, but not by hexamethonium (Ascher, 1972; Ascher, 1973; Berry & Cottrell, 1975). The inhibitory



receptor mediates an increase in potassium permeability (Ascher, 1972), and can be blocked by ergometrine (Ascher, 1972; Ascher, 1973; Berry & Cottrell, 1975). The stimulation of the presynaptic neurone can evoke a biphasic post-synaptic potential consisting of an EPSP followed by an IPSP (Berry & Cottrell, 1972; 1975).

Opposite receptors to glutamate have been described on neurones in the suboesophageal ganglion of the snail (*Helix aspersa*): the excitatory receptor is stimulated by kainic acid and quisqualic acid, whereas the inhibitory receptor is stimulated by quisqualic acid, but not by kainic acid (Walker, 1976). The cells containing both receptors respond in a biphasic fashion to glutamate: an initial inhibitory phase is followed by an excitation (Walker, 1976).

#### b. Vertebrates

Both excitatory and inhibitory receptors to 5-hydroxytryptamine (De Groat & Lalley, 1973) and to histamine (Brimble & Wallis, 1973) have been described on sympathetic ganglion cells.

Both excitatory and inhibitory receptors to acetylcholine have been described on single neurones in the mammalian CNS (see Krnjević, 1974; 1975). There are two kinds of excitatory receptor: <sup>a</sup> muscarinic receptor whose activation results in a

reduction in potassium permeability, and a nicotinic receptor mediating an excitatory response via an increase in both sodium and potassium permeabilities (Krnjević, 1975). The inhibitory acetylcholine receptor is muscarinic, and its activation results either in an increase in potassium permeability, or a reduction in sodium permeability (Krnjević, 1975). There is experimental evidence indicating that both excitatory and inhibitory acetylcholine receptors can co-exist on the same neurone. It has been reported that acetyl- $\beta$ -methylcholine, a muscarinic agonist, depresses neurones in the supraoptic nuclei of cats, whereas the same neurones are excited by nicotine; the depressant responses are antagonised by atropine, whereas the excitatory responses are antagonised by dihydro- $\beta$ -erythroidine (Barker *et al.*, 1971). A similar observation was made in the brain stem (Bradley & Dray, 1976): the same neurone could be excited by acetylcholine, but depressed by muscarine; nicotine was always excitatory.

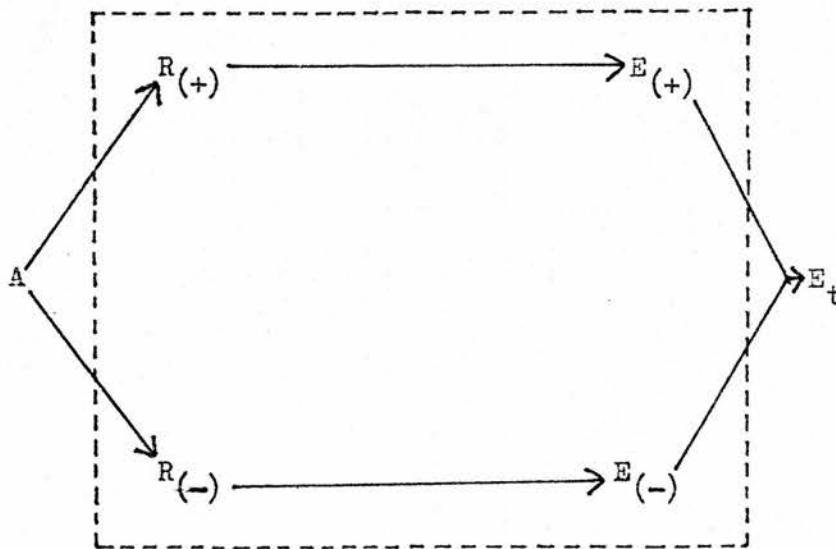
The monoamines noradrenaline, dopamine and 5-hydroxytryptamine applied by microelectrophoresis can evoke both excitatory and depressant responses on neurones in the mammalian brain (see Curtis & Crawford, 1969; Krnjević, 1974). A neurone can respond in a biphasic fashion to a monoamine: an initial depressant phase is followed by an excitatory phase suggesting that both inhibitory and excitatory receptors may co-exist on the same cell (see Chapter IV). The

experimental evidence presented in Chapter V suggests that noradrenaline can stimulate both  $\alpha$ - and  $\beta$ -adrenoceptors on cortical neurones, and that the  $\alpha$ -receptors mediate excitatory, and the  $\beta$ -receptors mediate depressant responses. There is also evidence suggesting that the functionally antagonistic  $\alpha$ - and  $\beta$ -receptors can co-exist on the same cell: the same cell can be depressed by a low dose of isoprenaline and salbutamol (stimulants of  $\beta$ -receptors), and excited by methoxamine and phenylephrine (stimulants of  $\alpha$ -receptors); a lower dose of isoprenaline evokes depressant responses (by stimulating  $\beta$ -receptors), whereas a higher dose causes excitation on the same cell (probably by stimulating  $\alpha$ -receptors as well); the antagonism of excitatory responses to adrenergic agonists by  $\alpha$ -adrenoceptor blocking agents can unmask an underlying depression, and antagonism of depressant responses by  $\beta$ -adrenoceptor blocking agents can reveal an excitatory response.

### A.3. THE MODEL

Ariëns et al. (1964b) used the term 'double agonism' in order to describe the phenomenon when 'a compound induces an effect by interaction with two different specific receptors but by means of a common effector'.

Antagonistic agonism can be defined as the special case of double agonism when the two receptors activated by the agonist mediate opposite effects. If it is assumed that the two kinds of receptor are functionally independent, the model described for "functional antagonism" can be applied (see Ariëns et al., 1964b). According to this model the total effect is obtained as the algebraic sum of the individual effects produced by the activation of each kind of receptor. This antagonistic agonism can be described by the following scheme:



In deriving equations for drug-receptor interaction at each of the receptors the same general postulates are used as described by van Rossum (1966). A further simplifying assumption which is made in the ensuing discussion is that no 'spare receptors' (Stephenson, 1956) are present in the

system. (When a receptor reserve exists for either of the two kinds of receptor, it has been argued that 'biological stimuli' rather than effects should be summated in order to derive the total effect [see van den Brink 1971; 1973] ).

The following notation is used in this Chapter:

A stands for the agonist;  $R_{(+)}$  and  $R_{(-)}$  are the opposite receptors;  $E_{(+)}$  and  $E_{(-)}$  are the effects produced by the interaction between drug A and  $R_{(+)}$  and  $R_{(-)}$  respectively;  $E_t$  is the total effect observed. (N.B. The subscripts (+) and (-) are used here to indicate the opposing nature of the two receptor populations; (+) does not necessarily mean 'excitatory', nor does (-) necessarily mean 'inhibitory'.)

### A.3.1. RELATIONSHIP BETWEEN AGONIST CONCENTRATION AND EFFECT

The interaction between agonist A and the opposite receptors  $R_{(+)}$  and  $R_{(-)}$  can be broken down into the following two steps:

1. Relationship between agonist concentration and effects (Ariëns et al., 1964a):

$$E_{(+)} = \frac{\alpha_{(+)} [A]}{K_{A(+)} + [A]} \quad (1a)$$

$$E_{(-)} = \frac{\alpha_{(-)} [A]}{K_{A(-)} + [A]} \quad (1b)$$

where  $\alpha_{(+)}$  and  $\alpha_{(-)}$  are the intrinsic activities of A on  $R_{(+)}$  and  $R_{(-)}$  respectively;  $K_{A(+)}$  and  $K_{A(-)}$  are the dissociation constants for the drug-receptor complexes  $AR_{(+)}$  and  $AR_{(-)}$  respectively;  $[A]$  is the concentration of the agonist.

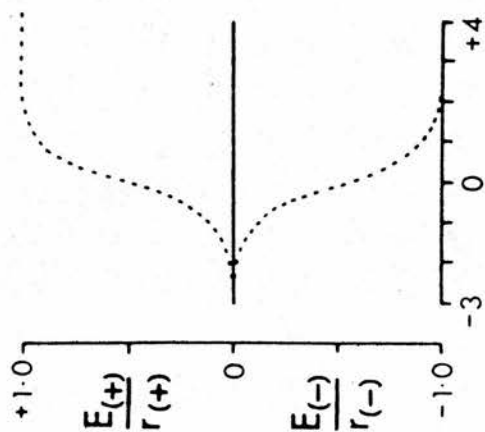
## 2. Summation of the effects:

$$E_t = E_{(+)} - E_{(-)}; \quad (2)$$

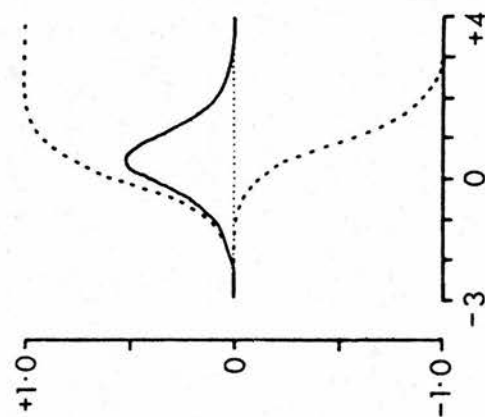
where  $E_t$  is the total effect.

As a concentration-effect curve is defined by the values of  $\alpha$  and  $K_A$ , the concentration- $E_t$  curve can be obtained if the relative values of  $\alpha_{(+)}$  and  $\alpha_{(-)}$ , and of  $K_{A(+)}$  and  $K_{A(-)}$  are known. On this basis, it is possible to distinguish between five different cases of agonist action. These five cases are illustrated in Fig. 1/Appendix. This figure shows for each of the five cases the relationship between agonist concentration  $[A]$  and effects  $E_{(+)}$  and  $E_{(-)}$  (broken lines), and also the resulting concentration-total effect curve obtained by the summation of the two individual curves (solid line). In calculating these curves, it was assumed, for the sake of simplicity, that the baseline activity of the test system is half-way between maximum inhibition and maximum excitation, i.e. the range of effect ( $r$ ) is the same in both directions:  $r_{(+)} = r_{(-)}$ . For the calculations of the curves, the agonist concentration was expressed as proportions of  $r_{(+)} = r_{(-)}$ .

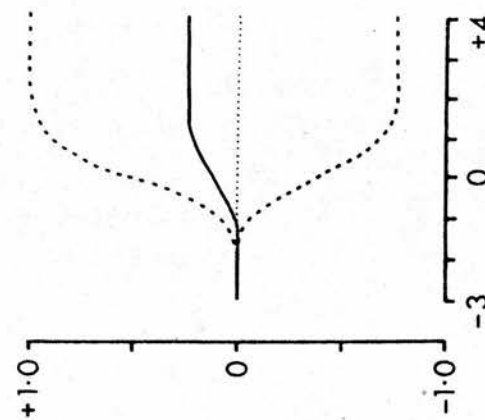
CASE 1



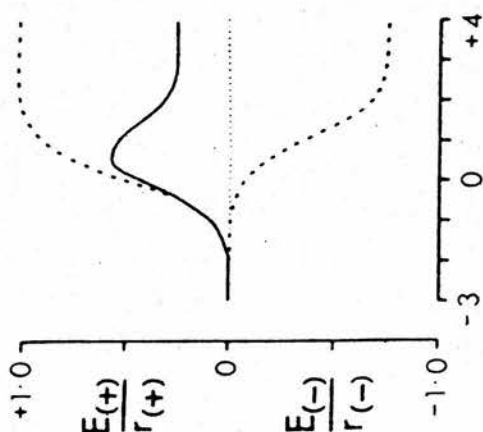
CASE 2



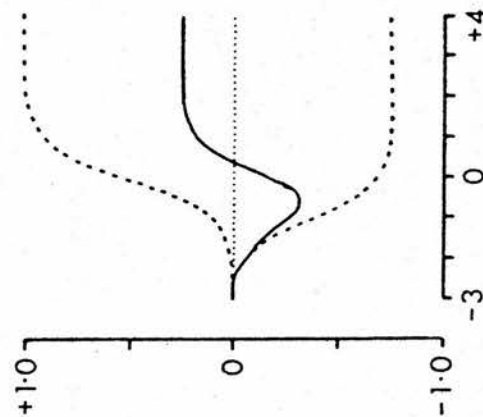
CASE 3



CASE 4



CASE 5



$\log([A]/K_{A(+)})$

Case 1:  $\alpha_{(+)} = \alpha_{(-)} ; K_{A(+)} = K_{A(-)}$

In this case, the two concentration-effect curves are mirror images of each other, and for any value of  $[A]$ ,  $E_{(+)} = E_{(-)}$ , and  $E_t = 0$ . Thus the functional antagonism is complete, and no response can be observed.

Case 2:  $\alpha_{(+)} = \alpha_{(-)} ; K_{A(+)} < K_{A(-)}$

In this case, the relationship between concentration and  $E_t$  is described by a monophasic bell-shaped curve. As  $[A]$  increases,  $E_t$  first increases up to a peak, and then declines asymptotically towards zero. The value of  $A$  which corresponds to  $E_{tmax}$  (the peak value of  $E_t$ ) may be called the 'reversal concentration' ( $[A]_r$ ) for the concentration-effect curve. Since at all values of  $[A]$   $E_{(+)} > E_{(-)}$ , the activation of  $R_{(+)}$  overrides the effect of the activation of  $R_{(-)}$ . Thus  $R_{(+)}$  can be called the dominant, and  $R_{(-)}$  the masked receptors (see IV.4.1.).

Case 3:  $\alpha_{(+)} > \alpha_{(-)} ; K_{A(+)} = K_{A(-)}$

In this case, the relationship between concentration and  $E_t$  is described by a monophasic saturation curve, in which  $E_{tmax} < r_{(+)}$ .



Case 4:  $\alpha_{(+)} > \alpha_{(-)}; K_{A(+)} < K_{A(-)}$

In this case,  $E_t$  first rises to a peak ( $E_{tmax}$ ), and then declines asymptotically to a plateau level.

Case 5:  $\alpha_{(+)} > \alpha_{(-)}; K_{A(+)} > K_{A(-)}$

In this case, a biphasic curve describes the relationship between agonist concentration and  $E_t$ . The first phase has a trough which is attained at  $[A] = [A]_r$ . The curve intersects the line representing the baseline activity of the system at a well-defined point. The agonist concentration value corresponding to this point can be called the 'iso-response' concentration ( $[A]_i$ ) since if  $[A] = [A]_i$ ,  $E_t = 0$ . If  $[A] < [A]_i$ ,  $E_{(-)} > E_{(+)}$ , and  $E_t$  is in the negative direction, indicating the dominance of  $R_{(-)}$ . If  $[A] > [A]_i$ ,  $E_{(+)} > E_{(-)}$ , and  $E_t$  is in the positive direction, indicating the dominance of  $R_{(+)}$ . Thus at  $[A] = [A]_i$  a switch in receptor dominance occurs.

The five cases of agonist action described above exhaust all the possibilities, since (+) and (-) are formally identical (either can stand for excitatory, or either for inhibitory). Thus the case where  $\alpha_{(+)} < \alpha_{(-)}$  and  $K_{A(+)} > K_{A(-)}$  is identical to Case 4.

### A.3.2. THE ACTION OF ANTAGONISTS

Let us consider how a drug B acting at  $R_{(+)}$  and/or  $R_{(-)}$  will change the interaction between drug A and  $R_{(+)}$  and/or  $R_{(-)}$  respectively, and how this is reflected in the concentration-effect curves shown in Fig. 1/Appendix. For the sake of simplicity, let us assume that  $\beta = 0$  (where  $\beta$  is the intrinsic activity of B), and thus B acts as a competitive antagonist of A (Ariëns *et al.*, 1964a). Thus the equation for competitive antagonism is applicable (Ariëns *et al.*, 1964a):

$$E'_{(+)} = \frac{\alpha_{(+)} [A]}{K_{A(+)} + [A] + K_{A(+)} \frac{[B]}{K_{B(+)}}} \quad (4a)$$

and

$$E'_{(-)} = \frac{\alpha_{(-)} [A]}{K_{A(-)} + [A] + K_{A(-)} \frac{[B]}{K_{B(-)}}} \quad (4b)$$

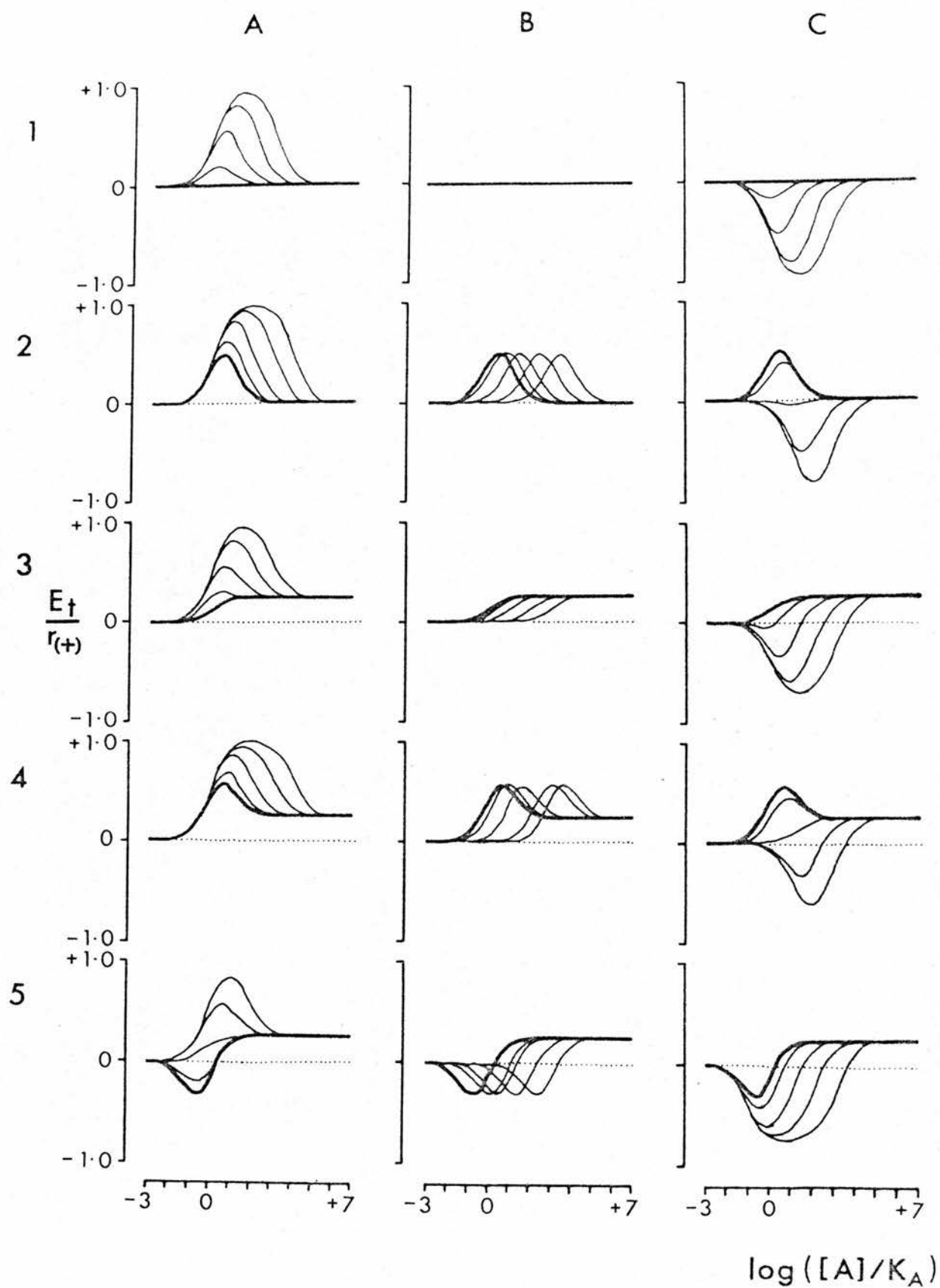
where  $E'_{(+)}$  and  $E'_{(-)}$  are the effects in the presence of B;  $K_{B(+)}$  is the dissociation constant for the antagonist-receptor complex  $BR_{(+)}$ ;  $K_{B(-)}$  is the dissociation constant for  $BR_{(-)}$ , and  $[B]$  is the concentration of antagonist B.

The total effect in the presence of antagonist B ( $E'_t$ ) will be:

$$E'_t = E'_t(+) - E'_t(-) \quad (5)$$

Equation (5) was used in order to calculate how the concentration-effect curves shown in Fig. 1 change in the presence of given concentrations of B (see Fig. 2/Appendix). Three types of competitive interaction are discussed.

Type A interaction:  $K_{B(+)} = \infty > K_{B(-)}$  (see Column A in Fig.2). In this type of interaction, the concentration-effect curve resulting from the interaction between A and  $R_{(-)}$  will be displaced to the right in a parallel fashion, whereas the concentration-effect curve resulting from the interaction between A and  $R_{(+)}$  will not change. The shift in the concentration- $E_{(-)}$  curve will result in an increase in  $E_t$  for all values of  $[A]$  (i.e.  $E'_t > E_t$ ). The observed pharmacological effect will depend on the algebraic sign of  $E_t$ . If  $E_t = 0$ , drug B will unmask a positive effect (1 in Column A); if  $E_t > 0$ , drug B will potentiate a positive effect (2, 3, 4 and 5 in Column A); if  $E_t < 0$ , drug B will antagonise or reverse a negative effect (5 in Column A).



Type B interaction:  $K_{B(+)} = K_{B(-)}$  (see Column B in Fig. 2).

In this type of interaction, the concentration- $E_{(+)}$  and the concentration- $E_{(-)}$  curves will be displaced to the right to the same extent by a given value of  $[B]$ , and this will result in a parallel shift of the concentration- $E_t$  curve to the right. In Case 1, where  $E_t = 0$ , drug B will not be able to unmask either a positive or a negative effect. In Case 3, the simple saturation curve will be displaced to the right, and thus  $E'_t < E_t$  at all values of  $[A]$ , i.e. the pharmacological effect of B will be antagonism of the positive effect. In Cases 2, 4, and 5, where the concentration- $E_t$  curves have ascending and descending limbs, a displacement of the curve to the right will result in an intersection between the concentration- $E_t$  and the concentration- $E'_t$  curves. The point of intersection can be defined as the value of  $[A]$  ( $[A]_{ia}$ , or 'iso-antagonism' concentration) at which  $E'_t = E_t$ . If  $[A] < [A]_{ia}$ ,  $E'_t < E_t$ , i.e. drug B antagonises the effect; if  $[A] > [A]_{ia}$ ,  $E'_t > E_t$ , i.e. drug B potentiates the effect (2, 4 and 5 in Column B). An increase in  $[B]$  will cause an increase in the value of  $[A]_{ia}$  (2, 4 and 5 in Column B), and also in the value of  $[A]_i$  (5 in Column B).

Type C interaction:  $K_{B(-)} = \infty < K_{B(+)}$  (see Column C in Fig. 2).

In this type of interaction,  $E'_t < E_t$  for all values of  $[A]$ .

The observed pharmacological effect will depend on the

algebraic signs of  $E_t$  and  $E'_t$ : 1. if  $E_t = 0$ , the effect of

B will be unmasking of a latent negative response (1 in Column C);

2. if  $E_t > 0$ , and  $E'_t > 0$ , the effect of B will be antagonism

of a positive response (2, 3, 4 and 5 in Column C); 3. if

$E_t > 0$ , and  $E'_t < 0$ , the effect of B will be reversal of a

positive response (2, 3, 4 and 5 in Column C); 4. if  $E_t < 0$ ,

the effect of B will be potentiation of a negative response

(5 in Column C). Increasing concentrations of B may produce

qualitatively different effects: 1. a lower concentration

of B may cause antagonism of a positive response; 2. a

higher concentration may cause reversal of a positive response;

3. a still higher concentration may potentiate the reversed response (2, 3 and 4 in Column C).

Competitive interactions of Types A, B and C are

extreme examples, but they can be used for deriving intermediate

types. If  $K_{B(+)} > K_{B(-)}$ , but  $K_{B(+)} < \infty$ , the effect of drug

B on the concentration-effect curves will be a combination

of Types A and B. Thus a lower concentration of drug B

may potentiate, whereas a higher concentration may antagonise

the response to a given concentration of drug A. Similarly,

if  $K_{B(+)} < K_{B(-)}$ , but  $K_{B(-)} < \infty$ , the effect of drug B on the concentration-effect curves will be a combination of Types B and C. Thus a lower concentration of drug B may antagonise, a higher concentration may reverse the response, whereas a still higher concentration may antagonise the reversed response.

#### A.4. APPLICATIONS OF THE MODEL

In this section the different cases of agonist and antagonist action described by the model are illustrated by experimental examples taken from the literature. Although alternative explanations might be found for some of the experimental findings referred to, the model has the advantage of offering a comprehensive framework for the interpretation of a wide range of agonist and antagonist effect.

##### A.4.1. UNMASKING OF LATENT RESPONSES

In Case 1 of antagonistic agonism no response to the agonist is obtained although the agonist activates both excitatory and inhibitory receptors (see Fig. 1/Appendix). The existence of receptor activation can be revealed by an antagonist which selectively blocks one kind of receptor (e.g. inhibitory), and thus unmasks a latent response resulting from the activation of the opposite type of receptor

(e.g. excitatory).

Thus the unmasking of latent excitatory adrenergic responses by  $\beta$ -adrenoceptor blocking agents, and of latent inhibitory responses by  $\alpha$ -adrenoceptor blocking agents has been described (Govier et al., 1966; Everitt & Cairncross, 1969; Persson & Johnson, 1970).

#### A.4.2. BELL-SHAPED DOSE-RESPONSE CURVES

Bell-shaped dose-response curves characterize Case 2 of antagonistic agonism. The role of the opposite receptors in these curves can be revealed either by the use of selective blockers of one receptor kind, or by the use of agonists acting only at one kind of receptor.

In the rat stomach fundus, the bell-shaped excitatory dose-response curve to adrenaline can be converted either into an excitatory saturation curve by propranolol, a blocker of inhibitory  $\beta$ -receptors, or into an inhibitory saturation curve by tolazoline, a blocker of excitatory  $\alpha$ -receptors (Tamayo et al., 1974). In the guinea pig vas deferens and in the human urinary bladder, the bell-shaped inhibitory dose-response curve to isoprenaline can be converted into an inhibitory saturation curve by phenoxybenzamine, a blocker of masked excitatory  $\alpha$ -receptors (Large, 1965; Awad et al., 1974).



In the guinea pig pulmonary artery, the bell-shaped dose effect curve to histamine can be converted into an inhibitory curve by mepyramine, a blocker of the dominant  $H_1$  receptors (Okpako, 1972). On neurones in the supraoptic nucleus, acetylcholine, acting at both excitatory nicotinic, and inhibitory muscarinic receptors, evokes an excitatory bell-shaped dose-response curve; the dose-response curve to nicotine is a simple excitatory saturation curve (Sakai et al., 1974).

Bell-shaped dose-response curves in themselves, however, do not necessarily indicate an action at opposite receptors: such curves can also result from auto-inhibition (Ariëns et al., 1964b) or desensitization (Bown et al., 1973; Frankhuijzen, 1975).

#### A.4.3. BIPHASIC DOSE-RESPONSE CURVES

A biphasic dose-response curve (cf. Case 5 of agonist action) to isoprenaline has been obtained in the guinea pig seminal vesicle and vas deferens (Spedding & Weetman, 1972); in the mouse spleen (Ignarro & Titus, 1968); and in the cat nictitating membrane (Trendelenburg, 1974). Similarly, biphasic dose-response curves to noradrenaline and adrenaline have been described in the rabbit heart

(Benfey & Varma, 1967). In all these cases, the first phase of the curve reflects the activation of  $\beta$ -, and the second phase reflects the activation of  $\alpha$ -receptors, indicating that isoprenaline, noradrenaline and adrenaline have a greater affinity for  $\beta$ - than for  $\alpha$ -receptors, and also that these agonists have a greater intrinsic activity at  $\alpha$ - than at  $\beta$ -receptors.

#### A.4.4. POTENTIATION BY ANTAGONISTS

The model predicts that the selective blockade of masked receptors will result in the potentiation of the response (see Column A in Fig. 2/Appendix).

In the case of opposite adrenoceptors, the potentiation of excitatory ( $\alpha$ ) actions by  $\beta$ -adrenoceptor blocking agents and of inhibitory ( $\beta$ ) actions by  $\alpha$ -adrenoceptor blocking agents is well documented (Large, 1965; Ignarro & Titus, 1968; Guimarães, 1969; Haffner et al., 1969; Persson & Johnson, 1970; DiSalvo & Fell, 1974; Trendelenburg, 1974). In the case of opposite histamine receptors, the potentiation of both excitatory  $H_1$  and inhibitory  $H_2$  actions of histamine by  $H_2$  and  $H_1$  receptor blocking agents, respectively, has also been described (Eyre, 1973; Glover et al., 1973; Goadby & Phillips, 1973; Parsons & Owen, 1973).

#### A.4.5. REVERSAL BY ANTAGONISTS

According to the model, the selective blockade of the dominant receptors may result in the reversal of the response (see Column C in Fig. 2/Appendix).

In the case of opposite adrenoceptors, the reversal of excitatory ( $\alpha$ ) actions by  $\alpha$ -adrenoceptors blocking agents, and of inhibitory ( $\beta$ ) actions by  $\beta$ -adrenoceptors blocking agents is well documented (Large, 1965; Diamond & Brody, 1966; Ignarro & Titus, 1968; Everitt & Cairncross, 1969; Guimarães & Osswald, 1969; Haffner *et al*, 1969; Persson & Johnson, 1970; Belisle & Gagnon, 1971). In the case of opposite histamine receptors, the reversal of both excitatory  $H_1$  and inhibitory  $H_2$  actions of histamine by  $H_1$  and  $H_2$  receptor blocking agents, respectively, has also been described (Okpako, 1972; Eyre, 1973; Goadby & Phillips, 1973).

#### A.4.6. THE DUAL ACTION OF ANTAGONISTS

An antagonist may have affinity for both the excitatory and inhibitory receptors (see Column B, Fig. 2/Appendix), but the affinity for one kind of receptor may be greater than for the other one. Such an antagonist may be called a 'dual antagonist'.

It is well documented that traditional  $\beta$ -adrenoceptor blocking agents block not only  $\beta$ -, but also  $\alpha$ -adrenoceptors, and that  $\alpha$ -receptor blockade occurs at somewhat higher

concentrations than is required for the blockade of  $\beta$ -receptors (Patil et al., 1968; Gulati et al., 1969). In systems which contain both  $\beta$ - and  $\alpha$ -receptors it has been demonstrated that low concentrations of  $\beta$ -adrenoceptor blocking agents can potentiate excitatory responses to adrenergic agonists, whereas higher concentrations of the same antagonists antagonise the responses (Burks & Cooper, 1967).

#### A.5. POSSIBLE FUNCTIONAL SIGNIFICANCE OF OPPOSITE RECEPTORS ON NEURONES

'Antagonistic agonism', resulting from the action of a neurotransmitter at both excitatory and inhibitory receptors on the same post-synaptic membrane, is a paradoxical phenomenon: it is difficult to see the immediate biological purpose of such a mechanism. Although there are indications that this phenomenon may exist in the mammalian central nervous system, it seems to be more prevalent in invertebrates, suggesting that we are dealing with a phylogenetically old arrangement. It is of interest that a similar organisation of receptors is common on smooth muscle cells where functionally antagonistic  $\alpha$ - and  $\beta$ -adrenoceptors have been known to exist since Dale's classical experiments with ergot (Dale, 1906; for review see A.2.).

#### A.5.1. THE TIME-COURSE OF NEURONAL RESPONSES

The action of a neurotransmitter at functionally masked receptors may influence the time-course of the response determined by the activation of the dominant receptors: the latency of the response will be elongated and the recovery phase will be shortened. Furthermore, the activation of masked receptors may create a contrast effect: if, for example, the dominant receptors are excitatory, the excitatory responses may be preceded and followed by inhibitory phases, resulting from the selective activation of the inhibitory receptors at low concentrations of the agonist.

#### A.5.2. SWITCH BETWEEN EXCITATION AND INHIBITION

The existence of both excitatory and inhibitory receptors to the same neurotransmitter substance on the same membrane creates a degree of plasticity for synaptic transmission: the net effect of the same transmitter may be excitatory or inhibitory in the same synapse depending on the relationship between the two kinds of receptor. Therefore, it is important to understand all those factors which may influence this relationship.

If the neurotransmitter evokes a biphasic dose response curve (see Case 5 of antagonistic agonism), the direction of the effect will depend on the dose applied: a low dose may be, for example, inhibitory, and a higher dose may be excitatory. This suggests that the amount of transmitter released from the pre-synaptic neurone may play a crucial role in determining the polarity of the post-synaptic potential.

The pre-synaptic neurone, however, can also modulate the relationship between the two opposite receptors in a different way: if the post-synaptic membrane is exposed to a higher concentration of the transmitter for a longer period, as may occur at higher firing frequencies of the pre-synaptic neurone, one kind of receptor may be selectively desensitised thus creating the possibility for the reversal of the response (Wachtel & Kandel, 1967). Thus, the same synapse may be excitatory at low levels of synaptic activity, and inhibitory at higher levels of activity (Wachtel & Kandel, 1967).

The relative contribution of each kind of receptor to the net effect may also depend on the level of activity of the post-synaptic cell. It has been reported that the sizes of the excitatory and inhibitory phases of biphasic responses to dopamine in ganglion cells in Planorbis depend on the membrane potential: as the cell is hyperpolarised, the excitatory phase increases and the inhibitory phase decreases (Berry & Cottrell, 1975). Similarly, the effects of exogenously applied monoamines on cerebral cortical neurones seem to be influenced by the baseline level of activity of the post-synaptic neurone: excitatory responses being more common at lower, and depressant responses being more frequent at higher firing rates (see IV.3.2.). These observations suggest the importance of other inputs to the post-synaptic cell in influencing the relationship between the two opposite receptors.

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